ASSIST
AUSTRALIAN SCHOOL SCIENCE INFORMATION SUPPORT FOR TEACHERS AND TECHNICIANS

GUIDELINES for best practice for microbiology in Australian schools

These guidelines were produced by the Science ASSIST project managed by the Australian Science Teachers Association (ASTA) in consultation with the Science Education Technicians Association (SETA). The Science ASSIST project is supported by the Australian Government Department of Education and Training through the Mathematics and Science Participation Program.

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Preface

Microbiology is an exciting and evolving area of science with links to many sections of the Australian Curriculum: Science. It is the study of biological organisms that are impossible to see individually with the naked eye.

Safety issues are a significant consideration as the subject is practical-based and there is potential for exposure to infectious organisms. It is the strict observance of correct procedures, which enables staff and students to work safely with microorganisms.

Australian schools are situated in geographical locations subject to a wide variety of local jurisdictional regulations as well as climatic conditions. They may be a part of a state/territory school jurisdiction that has strict guidelines for microbiology, or one that allows schools to make their own decisions based upon a risk management approach to activities conducted. Alternatively, a school may be part of a non-government sector or an Independent school that is responsible for developing its own policies and procedures.

These guidelines outline the underpinning knowledge and laboratory techniques required for schools to successfully prepare, deliver and disassemble microbiology practical activities.

Acknowledgements

The Australian Standard AS/NZS 2243.3.2010 *Safety in laboratories Part 3: Microbiological safety and containment* and the following related overseas documents, form the basis for developing this document.

- American Society for Microbiology. 2012. *Guidelines for Biosafety in Teaching Laboratories.* American Society for Microbiology website, https://www.asm.org/images/asm_biosafety_guidelines-FINAL.pdf
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(All documents were accessed in December 2016.)

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Contents

Chapter 1 Classification of microorganisms

Microbiology is the study of biological organisms that are impossible to see individually with the naked eye. Students should study microbiology because it has many applications in society, including in:

- the food industry.
- diagnostic microbiology—the study of disease in animal and plant species.
- the pharmaceutical industry—production of antibiotics and vaccines to treat disease.
- the biotechnology industry.

1.1 Beneficial uses of microorganisms

Some microorganisms are used to benefit the lives of humans and animals in areas such as food production, health and understanding disease. Some examples of beneficial microorganism use are:

Food production

- Yeast is used in the manufacture of beer and wine.
- Lactic acid bacteria are used to make fermented milk products such as yoghurt, sour cream, and buttermilk.

Medical

- Production of antibiotics and vaccines.
- Microorganisms used in biotechnology play a central role in recombinant DNA technology and genetic engineering.

Other associations

- The microbes that normally live in association with humans on the various surfaces of the body are called 'normal flora'. These microbes are known to protect their hosts from infections and otherwise promote good health.
- Microbes help purify wastewater in wastewater treatment facilities.
- Microbes help reduce atmospheric nitrogen and transform it to ammonia important for agriculture.[1]

1.2 Microorganisms detrimental to human health and environment

Many microorganisms are pathogenic, which means they are capable of causing disease.

Common examples of pathogenic bacteria include:

- *Salmonella*—causes food poisoning in humans typically through the ingestion of undercooked chicken or poor food hygiene practices.[2]
- *Legionella*—occurs naturally in soil and in untreated potting mix and grow well in water situated in cooling towers of air conditioning units and hot tubs[3]. It is potentially deadly to humans.
- *Staphylococcus aureus*—causes most staphylococcal infections such as skin infections, pneumonia, food poisoning, toxic shock syndrome and blood poisoning (bacteraemia).[4]

Some fungi can be pathogenic to some humans, for example, infectious agents such as the common mould *Aspergillus*. Whilst most people can breathe in these spores without effect, they can cause allergies, asthma and infections in people with weakened immune systems or lung disease.[5] *Candida* is a yeast-like fungus that occurs commonly as part of the normal flora

of the mouth, skin, intestinal tract, and vagina, but can cause a variety of infections. *Candida albicans* is the usual pathogen in humans.[6]

A very small number of species of Protozoa cause disease in people, for example, *Plasmodium species*, which cause malaria.[7]

When preparing to study microbiology, it is important to understand the different types of microorganisms, the hazards associated with them and the risk management strategies required.

1.3 Types of microorganisms

Microorganisms are very diverse and include the major groups of bacteria, fungi (moulds and yeasts), algae, protozoa and viruses. Many microorganisms are made up of only a single living cell (unicellular), some are multicellular and others do not have a true cellular appearance (acellular – viruses). When bacterial or yeast cells grow and multiply on agar plates they form colonies. These colonies can contain millions of cells.

Microorganisms are living cells and the main types studied in school microbiology include bacteria, fungi, and protozoa.

1.3.1 Bacteria

Bacteria are prokaryotic single celled organisms. They have a rigid cell wall, no nuclear membrane, no organelles in the cytoplasm, and have genetic material in the form of single continuous strands forming coils or loops. These are characteristic of all organisms in the kingdom Monera, including bacteria and blue-green algae.[8]

Typically, 1–2 micrometres in length, bacteria have a number of shapes, ranging from cocci (circular shape) to rods and spirals.

Bacteria can be found everywhere: on the palm of our hands, in our digestive system, on a laboratory bench, in the atmosphere. Some species of bacteria enable humans and animals to digest food, fight disease and protect from more debilitating diseases. Other bacteria will cause disease, which is often easily transmissible to others humans or animals.

1.3.2 Fungi

Fungi are eukaryotic unicellular or multicellular organisms. They have as their fundamental structural unit a cell type that contains a rigid cell wall, specialised organelles in the cytoplasm, a membrane-bound nucleus enclosing genetic material organised into chromosomes, no chlorophyll and an elaborate system of division by mitosis or meiosis, characteristic of all life forms except bacteria, blue-green algae, and other primitive microorganisms.[9]

Fungi include microorganisms such as yeasts and moulds. Some fungi are pathogenic to humans, and most are pathogenic to plants.[10]

Fungi have an essential role in the decomposition of dead and rotting soil and organic matter, and can also be found on foods in our kitchens. They have beneficial uses such as the production of antibiotics, as a leavening agent in bread production and as a direct food source such as mushrooms.

1.3.3 Protozoa

Protozoa are larger than bacteria, generally 10–52 micrometres, are unicellular eukaryotic microorganisms that lack a rigid cell wall. They have been found in almost every kind of soil

environment from peat bogs to arid desert sands. They teem in the deep sea as well as near the surface of waters, and can be found even in frigid Arctic and Antarctic waters.

Some species of protozoa are part of the normal microbial flora of animals, and live in the guts of insects and mammals, helping to break down complex food particles into simpler molecules. Others are parasitic, causing human diseases such as malaria, sleeping sickness and dysentery.

1.4 Classification of microorganisms by risk group

The World Health Organisation (WHO) has produced an extensive document on biosafety and has recommended that countries draw up classification of microorganisms within their boundaries according to the degree of risk considering:

1. pathogenicity of the agent
2. the mode of transmission and host range of the organism
3. local availability of effective preventative measures
4. local availability of effective treatment.[11]

The Australian Standard AS/NZS 2243.3.2010 *Safety in laboratories Part 3: Microbiological safety and containment* has drawn up the following classification for microorganisms that are infectious for humans by risk group.

1.4.1 Risk Group 1

Risk Group 1 (RG1) includes those microorganisms that are unlikely to cause human or animal disease in healthy adult humans.

The AS/NZS 2243.3:2010 *Safety in Laboratories – Microbiological safety and containment* does not list Risk Group 1 microorganisms, as there are too many. Some examples used in Australian schools are *Escherichia coli (K-12)*, *Staphylococcus epidermidis, Micrococcus luteus, Bacillus subtilis, Saccharomyces cerevisiae* and *Penicillium chrysogenum*.

1.4.2 Risk Group 2

Risk Group 2 (RG2) includes those microorganisms that are unlikely to pose a significant risk to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause infection, but effective treatment and preventative measures are available, and the risk of spread is limited.

Examples of microbes that are classified risk group 2 include: *Bordetella pertussis* (causative agent of whooping cough), *Chlamydia spp.* (a common sexually transmitted disease) and *Vibrio cholerae* (cause of epidemic Cholera).

1.4.3 Risk Group 3

Risk Group 3 (RG3) includes those microorganisms that usually cause serious human or animal disease and may present a significant risk to laboratory workers. They could present a limited to moderate risk if spread in the community or the environment, but there are usually effective preventative measures or treatments available.

Examples of microbes that are classified Risk Group 3 include: *Bacillus anthracis* (the etiological agent of Anthrax), *Coxiella burnetii* (the causative agent of Q fever) and *Yersinia pestis* (the causative agent of plague).

1.4.4 Risk Group 4

Risk Group 4 (RG4) includes those microorganisms that usually produce life-threatening human or animal disease, represent a significant risk to laboratory workers and may be readily transmissible from one individual to another. Effective treatment and preventative measures are not usually available.[12]

Examples of microbes that are classified risk group 4 include: Ebola virus (cause of haemorrhagic fever) and Lassa virus (cause of Lassa haemorrhagic fever).

Chapter 2 Classification of laboratories

2.1 Working with microorganisms—Physical containment levels

Microorganisms vary in their capacity to infect humans, however, it is considered good practice to consider all microorganisms as potential pathogens and use a combination of strategies to handle them safely. These include

- structures such as building designs
- engineering functions such as air pressure controls
- specialist equipment such as biological safety cabinets
- standard microbiological techniques.

Physical containment is the term used to describe procedures and structures designed to reduce or prevent the release of viable organisms into the outside environment.[13] In Australia, laboratories are classified into four levels of Physical Containment (PC 1–4). The level of containment must be at least the level appropriate for the risk group of the microorganism being used in the laboratory i.e. PC1 for RG1, PC2 for RG2 etc.

2.1.1 Physical Containment Level 1 (PC1)

A Physical Containment Level 1 laboratory is suitable for work with material likely to contain microorganisms that are classified as Risk Group 1 microorganisms. They require no special containment equipment and are suitable for schools and undergraduate teaching laboratories. Work may be carried out on the open bench as long as hazard levels are low and standard microbiological techniques are followed in order to protect laboratory personnel. Specimens that have been fixed or inactivated may be handled in a PC1 facility.[14]

2.1.2 Physical Containment Level 2 (PC2)

A Physical Containment Level 2 laboratory is suitable for work with material likely to contain microorganisms that are classified as Risk Group 2 microorganisms. If working with specimens containing microorganisms transmissible by the respiratory route or if the work produces a significant risk to humans or the environment from the production of infectious aerosols, a biological safety cabinet must be used.

2.1.3 Physical Containment Level 3 (PC3)

A Physical Containment Level 3 laboratory is suitable for work carried out with microorganisms or material likely to contain microorganisms that are classified as Risk Group 3 microorganisms. A PC3 laboratory or facility provides additional building features and services to minimise the risk of infection to individuals, the community and the environment.

2.1.4 Physical Containment Level 4 (PC4)

A Physical Containment Level 4 laboratory is suitable for work with microorganisms classified as Risk Group 4 microorganisms. A PC4 laboratory or facility is situated in a building separate from other laboratories facilities or constructed as an isolated area within a building.[15]

2.2 Australian school facilities

Australian school science laboratories are generally constructed to Physical Containment 1 (PC1), the most basic level. At this level, the laboratory is suitable for work with microorganisms where the hazard levels are low, and where laboratory or facility personnel can be adequately protected by standard microbiological techniques.[16] Standard

microbiological technique is where staff and students are proficient in methods to contain any uncontrolled spread of microbes in order to protect:

- practical investigations from becoming contaminated with microbes from external sources
- the operators (students, teachers and technicians) from the very small possibility of infection.[17]

Laboratory design considerations for work with Risk Group 1 microorganisms in PC1 labs.

- Laboratory floors, benches and seating must be made from smooth impervious material to ensure they are easily cleanable, easily decontaminated and resistant to damage by the cleaning agents and/or disinfectants that will be used in the laboratory.

  Note: Laboratories that have carpet covering any part of the floor is not deemed to be suitable as a PC1 laboratory. Carpets can harbour fungal spores and bacteria, even when an activity is taking place several metres away.

- Bench top surfaces must also be made of heat resistant material.
- Sink for hand washing with potable hot and cold water services shall be provided inside the laboratory near the exit.
- Designated storage or hanging facilities for protective clothing must be available within the facility.
- Open spaces between and under benches, cabinets and equipment shall be accessible for cleaning.
- Internal fittings and fixtures must be arranged to minimise dust accumulation.
- Provision of an eyewash and safety shower area.

Schools wishing to conduct activities using RG1 microorganisms should assess their own facility for compliance with the requirements of AS/NZS 2243.3 (Section 2.5)

If school facilities allow, a specialised microbiology laboratory could be considered for microbiology activities. Select a laboratory that is easily accessible from the preparation room, has facilities at Physical Containment 1 level, and have little or no through foot traffic. Equipment such as an incubator may be stored and used in this laboratory if it is not likely to be interfered with by other students.

However, it is safe to use the same room as a microbiology laboratory and a teaching room for other classes. When not in use and whilst non-microbiology classes are in session, all cultures should be stored properly (e.g., in incubators or refrigerators) and not left out in common areas. Cultures and waste should be decontaminated immediately or stored in a separate, non-shared location that is not accessible by students. Laboratory benches should be disinfected immediately prior to and following microbiological activities to prevent contamination of microbiology work and subsequent student activities.

Chapter 3 Microbiology in Australian schools

Australian school jurisdictions permit the use of microorganisms in Risk Group 1 where there is a low risk of disease to students, teachers and laboratory technicians. The use of Risk Group 1 microbes in schools does not negate the importance of good microbiological practice.

It should be noted that even though microorganisms are from RG1, some could still pose a low level of risk to the community, as they can be capable of causing disease if provided with appropriate conditions (referred to as opportunistic). People who are immunocompromised or immunosuppressed are at greater risk, and along with those who are pregnant or may become pregnant, should seek advice from their medical professional before embarking on microbiological activities.

It is good practice to regard all microorganisms, regardless of their risk group, as potential pathogens and to handle them with standard microbiological techniques to minimise risk to laboratory staff, students and the environment.

Microbiological activities can be safely undertaken in schools, provided proper facilities are in place and precautions are identified and implemented. These include:

- appropriate risk assessment processes
- consideration for the microorganisms to be used (see Chapter 1)
- correct laboratory facilities (see Chapter 2)
- standard microbiological techniques (see Chapter 4).

3.1 Risk assessment

It is a legal requirement for every workplace to manage risks to health and safety so far as is reasonably practicable. It is a continuous process of identifying hazards, assessing and controlling risks that may affect the functioning of a workplace.[18]

A biological risk assessment of a school microbiology activity needs to ensure that the risks are not underestimated or there could be undesirable consequences. Living cells have the capacity to multiply in optimum conditions and all microorganisms should be considered as if they are pathogens. Incorrect handling of microorganisms can cause laboratory-acquired infections. Therefore, it is vital that due consideration is given to the type of microorganism being used as well as the procedures being implemented. A risk assessment should be used to alert staff and students to the hazards of working with infectious agents and for the need for developing proficiency in the use of selected safe practices and equipment.

According to *Biosafety in microbiological and biomedical laboratories* (BMBL)[19], the following five steps should be considered:

1. **Identify agent hazards and perform an initial assessment of risk**

   List all known microbes used, the hazardous characteristics of a known infectious or potentially infectious agent or material and the likely route of transmission (via skin, eyes, hand to mouth, nasal inhalation etc.), consider if any wild or unknown microbes will be introduced. Also, consider the susceptibility of certain individuals in the laboratory to infection from the microorganism.

2. **Identify laboratory procedure hazards**

   List all procedural hazards that can result in a person's exposure to an agent, including equipment used, the complexity of the task and the laboratory environment, including decontamination processes.

3. **Make a determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment**

   Determine control strategies by considering the laboratory facilities, techniques and processes used to prevent exposure.

4. **Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment**

   Evaluate the technical proficiency of staff and students—their ability to control hazards, the presence of good microbiological habits and implement control measures, eliminate, substitute, engineering controls, administrative control, personal protective equipment (PPE). Also, consider the behaviour of the students.

5. **Review the risk assessment with a biosafety professional, subject matter expert, and the IBC.**

   Review the risk assessment with a person knowledgeable in biosafety practices before work proceeds. After the activity, monitor, review and document the effectiveness of the control measures.

Before schools embark on working with microorganisms they should ask the following questions and perform a site-specific biological risk assessment.

- What microorganism is being used? Is it a Risk Group 1 microorganism?
- Do the school facilities comply with the requirements of Physical Containment Level 1 laboratories?
- Does the school have the necessary equipment for sterilisation and decontamination procedures?
- Does the staff have training in microbiological skills?
- What manipulations are being performed with the microorganism? Are methods being used to eliminate or minimise exposure to potentially infectious material via aerosols, splashes, ingestion, absorption and accidental inoculation?
- Are any staff or students wishing to participate in microbiological activities immunocompromised or immunosuppressed (include those who are pregnant or may become pregnant, or are living with or caring for an immunocompromised individual)? These individuals are more prone to infections and they should consult a doctor to determine whether their participation is appropriate.

The AS/NZS 2243.3:2010 *Safety in Laboratories – Microbiological safety and containment* states:

‘*2.6.5 At-risk persons*

> *Persons who are immunosuppressed, immunocompromised, or otherwise unduly vulnerable to infection, such as persons who are diabetic, should inform their supervisor or person responsible for microbiological safety of their condition so that appropriate action may be taken. Some microorganisms that are regarded as part of the normal flora of humans or animals may be pathogenic for immunocompromised persons.’*

3.2 School work levels

Three UK organisations: Society for General Microbiology (SGM), Microbiology in schools advisory committee (MISAC) and CLEAPSS, have categorised microbiology activities conducted in schools into three levels.

Science ASSIST has adapted these levels, by subdividing the UK level 2 to create an additional level for application to the Australian context.

Note: These four levels are not the same as levels of containment, but guidelines for suitable activities conducted in schools. Each higher level includes the microorganisms used in lower levels.

Safety Note: All microbiology work with students, requires close and constant supervision at each level

Level 1 [Very low risk] Observation and growth of certain microorganisms

- **Microorganisms:** which have little, if any, known risk.
  - **Limited to algae, yeasts, moulds and bacteria used for food purposes**, some moulds and commonly occurring bacteria where they grow naturally on decaying vegetable material e.g. brewer's or baker's yeast and certain protozoa or moulds, such as bread mould.
  - These microorganisms to be cultured on the substances on which they grow naturally in closed containers at the ambient room temperature, except for culturing yogurt at 43°C
  - No microorganisms associated with meat or fish.
  - No agar plates
- **Staff training:** No specialist training required for teacher or technician.
- **Facilities:** No special facilities required or need for a steriliser.
- **Waste:** No specialist treatment: Able to be placed into the regular waste or put down the sink.

Level 2 [Low risk]: Simple environmental sampling

- **Microorganisms**: sourced from the environment where the risk of procuring human pathogens is unlikely.
  - Cultured only in closed containers on nutrient agar plates or broth at a maximum of 30°C. The only exception is the sampling of airborne particles for the Pasteur broth activity
  - No subculturing of cultures.
  - Nutrient or plain agar/broth to be used where appropriate.
- **Staff training:** Staff to be trained in sterilisation and decontamination procedures.
- **Facilities:** PC1 facilities required. Autoclave or pressure cooker required for sterile preparation of agar/broth etc. and for decontamination.
- **Waste:** to be sterilised before disposal.

Level 3 [Low-medium risk]: The growth and subculture of pure cultures of microorganisms

Note: schools should check whether level 3 activities are permitted in their school jurisdiction, as certain subculturing is prohibited in some jurisdictions

- **Microorganisms** sourced from school biological suppliers and limited to
  - Certain protozoa (including slime moulds, e.g. *Physarum polycephalum*)
  - Risk Group 1 Fungi and bacteria (Low risk—not associated with disease in healthy adult humans)
  - Risk assessment to consider immunosuppressed staff and students.
  - No subculturing from plates or broths inoculated by students.
- **Staff training:** Staff to be highly trained

- in aseptic technique and recognise contamination of pure cultures.
- to subculture pure samples obtained from reputable suppliers for student use.
- Additional staff recommended for student supervision.

- **Facilities:** PC1 facilities required. Autoclave or pressure cooker required for sterile prep of agar/broth etc. and for decontamination.
- **Waste:** to be sterilised before disposal.

Level 4 [Medium-high risk]: Advanced work in subculturing and manipulations

This level is beyond the scope of this document

It is expected that microorganisms, facilities and waste guidelines will be similar to Level 3 with staff highly trained in microbiology and the manipulations required

3.3 Choice of microorganism

Science ASSIST recommends that schools choose the microorganism with the lowest level of risk that will meet the learning outcomes. Schools need to ensure that they have the required facilities and equipment and the necessary staff training to be able to manage the risks.

When considering microorganisms for Level 3 work, table 1 suggests a number of Risk group 1 microorganisms that are readily available from reputable Australian suppliers. See Science ASSIST *School science suppliers list* https://assist.asta.edu.au/resource/664/school-science-suppliers. These microorganisms are suitable for use in the activities included in this guideline.

Table 1: Risk group 1 microorganisms suitable for use in Australian schools

Microorganism	Gram stain	Shape	Description	Habitat
Bacillus subtilis	positive	Bacillus (rod shaped)	4-10µm long and ~0.25-1µm diameter Singles or in chains	soil air, water, animals
Escherichia coli K-12 strain*	negative	Bacillus (rod shaped)	~ 2µm long and ~0.25-1µm diameter Single cells	gastro intestinal tract of humans and animals
Micrococcus luteus	positive	Coccus (spherical)	1.0-1.8µm diameter irregular clusters, tetrads or pairs	mammalian skin, soil
Mucor – positive and negative strains	n.a.	Hyphae, sporangiophores, some branched ending in round sporangia, sporangiospores	filamentous (long thread-like structure)	soil, plants, decaying fruit and vegetable matter
Penicillium chrysogenum **	n.a.	Hyphae, branched conidiophores, conidiospores	filamentous (long thread-like structure)	blue-green mould on stale bread, fruit and nuts. Used in production of green and blue mould cheese

Physarum polycephalum	n.a.	Slime mould	bright yellow mass of multinucleate protoplasm	under the bark of decaying trees and amongst leaf litter on the forest floor
Saccharomyces cerevisiae	n.a.	Yeast: oval shaped cells with budding	globular shaped green yellow	gastrointestinal tract, body surfaces of insects and warm-blooded animals. Used in fermentation of food.
Staphylococcus epidermidis	positive	Coccus (spherical)	0.5–1.5µm diameter cocci that usually form in clusters	skin, mucous membranes

* *E. coli* K12 strain is considered a low risk of infection due to absence of virulence genes [20]

** *Penicillium chrysogenum* is considered suitable for use in schools however, it is important to consider that this strain may produce a large number of spores that cause allergies and asthma attacks in some users. This fungus should be handled before sporulation occurs.[21] Further information can be found at http://www.misac.org.uk/PDFs/MiSAC_suitable_and_unsuitable_micro-organisms.pdf

3.4 Science ASSIST student practical activities and SOPs

These SOPs are located in Appendix 1.

3.4.1 Student activities suitable for level 1:

SOP: Examining life in pond water

Life in pond water consists of small animals that can be viewed macroscopically or microscopically. Different samples of water consist of differing animals from different habitats. This activity looks at how living things satisfy their needs for food, water and air.

SOP: Fermentation of yeast

Yeast requires specific conditions in which to ferment and become active. When used in food production particularly bread making, the yeast must be at a certain temperature to grow and make the bread rise. This activity examines the fermentation of yeast and asks ‘What is the best temperature for yeast to grow?’.

SOP: Making yoghurt

Yoghurt is a fun way to look at the use of microbes in food production.

SOP: Examine mushroom spores

Fungal spores are microscopic. However, we can see the pattern they make on paper when they are released from the gills of the mushroom by making a spore print.

SOP: Growing fungi on bread

Bread mould is a simple fungus, which derives its food from a variety of materials such as grains, fruits, vegetables or flesh. Mould spores are tiny and usually remain suspended in air. As soon as it finds the right environment for it to grow, the spores transform into the living fungus.

3.4.2 Student activities suitable for level 2

SOP: Microbes are everywhere

This is a microbiological activity to gain techniques in environmental sampling and to look at microbes in the environment around us.

SOP: Pasteur's experiment

An investigation to demonstrate Pasteur's experiment that microbial life does not spontaneously generate in sterile nutrient broth.

SOP: Preparing agar plates

A step-by-step guide to preparing and sterilising nutrient and plain agar for aseptic preparation of agar plates and broths. The science technician usually prepares agar plates, however this is also a suitable activity for students.

3.4.3 Student activities suitable for level 3:

SOP: Physarum polycephalum care and use

Physarum polycephalum is a slime mould that is easily demonstrated to show growth patterns and cytoplasmic streaming. Students can be involved by feeding the slime mould oat flakes.

SOP: Preparing a bacterial lawn culture

Aseptic technique is an important technique to use when preparing for and undertaking any microbiological procedure. In this activity, students use aseptic technique to lawn inoculate an agar plate

SOP: Streak plate inoculation

Inoculating a culture media with an inoculating loop to obtain isolated colonies.

SOP: Susceptibility testing of antiseptics and disinfectants

This activity uses aseptic technique and culture inoculation techniques to test the effectiveness of antiseptics and disinfectants on bacteria.

SOP: Gram stain a microbial culture smear

Gram stains are used to differentiate and identify different bacteria. This activity involves the preparation of a culture smear on a slide, the Gram stain and subsequent microscopic examination of the stained smear

Chapter 4 Safety in the school laboratory—protocols

Systems and protocols for safety during microbiological activities need to be planned and implemented prior to commencing laboratory activities. Teachers and laboratory technicians must enforce standard microbiological techniques including aseptic techniques, microbiology laboratory rules and spills procedures in order to maintain safety.

It is imperative that procedures for microbiological safety be followed closely. This includes the prevention of transmission of disease caused by poor microbiological technique. The most probable routes of transmission in school microbiology are:

- direct skin, eye or mucosal membrane exposure to an agent
- inhalation of infectious aerosols.[22]

Many cases of laboratory-acquired infections can be prevented by the use of good microbiological technique acquired through training programmes.

4.1 Training of staff

Teachers and laboratory technicians undertaking microbiological procedures should receive appropriate training prior to commencing this work unless they have prior knowledge, skills and experience. Staff need to demonstrate good microbiological technique, competency and confidence when performing procedures, interpretations and outcomes of activities, and competency in microbiological hazard awareness to maximise the student experience in microbiology. Staff who have completed microbiological training from higher educational facilities and/or relevant workplaces generally have the skills and knowledge sufficient for the school setting.

Good microbiological technique requires an understanding of aseptic procedures and the strict adherence to working methods designed to eliminate or minimise exposure to potentially infectious material via aerosols, splashes, ingestion, absorption and accidental inoculation.[23]

Training should be provided for staff who will then be able to teach students. The trainer should be a person skilled and experienced in microbiology. Alternatively, there are external training providers (and some universities) who can deliver this training.

Science ASSIST recommends the following training practices for consideration by Australian schools. The practices are adapted from *Guidelines for Biosafety in Teaching Laboratories* published by American Society for Microbiology (2012).

Comprehensive microbiology training should achieve the following outcomes:

- The trainer provides extensive initial training for teachers and laboratory technicians to:
    1. cover the safety hazards of working with microorganisms
    2. develop competency in microbiological practices and techniques required for work in Physical Containment 1 (PC1) laboratories using Risk Group 1 (RG1) microbes.
    3. develop skills and confidence with these practices and techniques to be able to demonstrate to students how to handle microorganisms safely and responsibly minimising hazards and risks.
- Provide refresher training annually and/or whenever a new procedural change is required.
- Ensure staff and students are aware of rules and regulations in their jurisdiction relating to the handling and cultivation of microorganisms.

- Ensure staff are competent in carrying out risk identification, assessment and management of controls, and to document these in a risk assessment.
- Emphasise to students the importance of reporting accidental spills and exposures.
- Keep a biosafety manual specific to the laboratory and/or unit of work in the laboratory.

Records of successfully completed staff training should be established and maintained within the school.

4.2 Microbiology laboratory rules

Working safely in microbiology necessitates that staff and students be aware of, and follow, microbiology laboratory rules and specific work practices. These are in addition to laboratory safety rules followed in general science laboratories. The following work procedures are the minimal requirements for a school microbiology laboratory. Each school should develop its own policy in regards to microbiology laboratory rules.

- Science ASSIST strongly recommends that all science rooms be locked unless a teacher or other authorised person is present.[24] Laboratory access by students and visitors should be under supervision only.
- Since microbiology activities are not conducted in every laboratory all year round, Science ASSIST suggests that a sign only be displayed at the entrance to the laboratory and/or preparation rooms when microbiological agents are being handled, particularly for school level 3 activities. The sign should be removed when biohazards are no longer present. This sign should include the biohazard symbol and laboratory containment level. Any access restrictions, plus contact information for responsible persons, should also be included.
- A biohazard symbol should also be displayed on equipment such as incubators, fridges, freezers or containers when microorganisms are being stored. Again, this can be removed when the biohazard material has been removed.

  The AS/NZS 2243.3:2010 *Safety in Laboratories – Microbiological safety and containment* states:

  '5.2 REQUIREMENTS FOR PC1 LABORATORIES

  5.2.1 General

  'A sign complying with Appendix D showing the level of containment, together with hazard symbols as appropriate and any access restrictions should be prominently displayed at the entrance.'

  The biological hazard symbol per Appendix D is a black symbol on a yellow background. General microbiology laboratories should also have the laboratory containment level indicated on the sign in black letters as below:

- Do not commence work unless a site-specific risk assessment is completed and control measures are implemented.
- Mouth pipetting is prohibited.
- Eating, drinking, and the application of cosmetics are prohibited.
- Storage of food and drink in laboratory refrigerators is prohibited.
- PPE will comply with AS/NZ 2243:1. Lab coats or disposable aprons, which protect the front of the body should be worn. Long hair must be tied back.
- Hands must be thoroughly washed before commencing activities and before leaving the laboratory.
- Gloves are worn if there is a cut or wound on the hand, and discarded after use with infectious laboratory waste. The cut or wound should also be covered with a band-aid or other dressing.
- All bench surfaces should be disinfected with 70% ethanol prior to and after handling microorganisms.[25]
- Precautions must be taken to ensure reading and writing materials do not become contaminated.[26] Keep the work area free of non-essential materials.[27]
- Good laboratory hygiene must be maintained and aseptic technique should be used when culturing microorganisms.
    - Do not breathe on cultures of agar plates. Keep conversation to a minimum.
    - Do not touch face or other parts of body.
    - Do not touch contaminated areas such as windows, doorknobs, seats, benches etc. with sterile equipment such as bacteriological loops.
- Cultures must be clearly identified, dated and appropriately stored, and should remain on open benches no longer than is necessary.
- Aerosols must be controlled to minimise their production especially on open benches. When using a loop, draw it from the cooler to the hottest parts of the Bunsen burner flame or use a hooded or electric Bunsen burner.
- Prior to incubation, Petri dishes should be sealed and incubated under aerobic conditions.
- Laboratory waste contaminated with microorganisms must be decontaminated prior to disposal in either an autoclave or appropriate pressure cooker.
- Work benches must be decontaminated immediately following spills.
- Communally used items and furniture such as doors, cupboards, telephones and keyboards must be regularly cleaned and disinfected.
- Staff and students must remove laboratory coats and thoroughly wash their hands before moving to areas outside the laboratory.

While conducting their experiments, students should never contaminate items that will leave the laboratory. The area for culturing and working with microorganisms should be as separate as possible from the area for taking notes or using laptops. Students should write with laboratory-use-only pens and pencils during a practical activity. Always minimise the number of notebooks and/or lab manuals on the lab bench. All other personal belongings should be stored away from the work area in spaces approved by the teacher. Papers that students will take home should be protected from contamination during the lab period. Optional approaches for taking notes in the lab are dependent upon the design of the facility, and practices will vary from school to school.

Instructions for practical activities should be laminated pages that are disinfected after each class and remain stored in the lab. A desk copy can be kept at each lab table for use during class. Alternatively, practical activity instructions can be displayed from the teacher's laptop via data projector for class use.

These microbiology laboratory rules and specific work practices form part of a staff training package and students should understand these rules before commencing any microbiological studies. These rules can be laminated and displayed in each microbiology laboratory. A printable version is in the Appendix 3 of this guideline.

4.3 Hand washing and hygiene

Hand washing is an integral part of establishing a clean microbiology working environment, and an important hygiene practice when working in the laboratory. Hand washing must be performed thoroughly prior to and after any microbiological activity.

AS2243.3: 2010 recommends the use of Chlorhexidine (0.5-40% w/v) in alcoholic formulations for 2 minutes for skin disinfection. In the context of a school laboratory handling RG1 microbes, commercially available anti-bacterial liquid hand wash is an option and is preferable to soap. Schools should consult the relevant safety data sheet when selecting a suitable hand wash product.

Liquid hand wash pump packs should be placed at sinks designated for hand washing. A hand-washing sink should be provided in each laboratory, preferably near the exit door. Elbow or foot operated tap handles should be considered when planning new or refurbishing laboratories.

Paper towel should be used in preference to reusable cloth towels. Paper towel dispensers should be located as close as practicable to the hand-washing sink. Used paper towel should be collected in a waste bag for disposal to an industrial waste bin. Hands should be dried thoroughly to ensure no contaminants remain in the cleansing liquid.

A printable version of hand wash technique published by the World Health Organisation is available at http://www.who.int/gpsc/5may/How_To_HandWash_Poster.pdf

4.4 Protective clothing and equipment

Personal protective equipment (PPE) refers to a variety of barriers, used alone or in combination, to protect mucous membranes, airways, skin and clothing from contact with infectious agents. PPE used as part of standard precautions includes aprons, gowns, gloves, surgical masks, protective eyewear and face shields.[28] A site specific risk assessment will highlight items of PPE that are required to minimise hazards in school microbiology.

- Personal protective equipment required for use in school PC1 labs using RG1 microbes:
    - Single-use plastic aprons that provide protection to the front part of the body shall be worn within the laboratory. Disposable aprons should be disposed of immediately after the microbiological activity in a contaminated waste bag to be double bagged and disposed in an industrial bin.
    - Closed leather footwear shall be worn.
    - Safety glasses or goggles shall be worn to protect eyes from splashes and other hazards.
- Gloves should only be worn if the person has any cuts or skin problems such as dermatitis. They could obstruct some activities and create other hazards if using a Bunsen burner. Gloves are not required for standard microbiological procedures if proper hand hygiene is performed. Proper hand hygiene involves thorough hand cleansing prior to and immediately after finishing handling microorganisms and any time that microbes accidentally contact the skin.[29]

Safety glasses are intended to provide eye protection against common laboratory hazards such as chemical and biological splashes and low impact energy activities. The lenses must be appropriate to the protection required. Note while it is possible to obtain prescription safety glasses, normal prescription glasses are not considered safety glasses as they do not provide protection against splashes.[30] Safety glasses should be cleaned regularly according to the manufacturer's instructions, generally with detergent solution, and be completely dry before being stored.

Staff and students should don PPE in the following order:

1. apron or lab coat
2. safety glasses
3. disposable gloves, if required

PPE must be removed before leaving the laboratory in the following order and stored within the laboratory.

1. disposable gloves, if used. (For 'Removal of gloves technique' see http://education.qld.gov.au/health/pdfs/healthsafety/handsgloves.pdf)
2. safety glasses
3. apron or lab coat

Perform hand hygiene immediately after removing PPE.

Chapter 5 A clean environment—decontamination and sterilisation

5.1 Importance of a clean work space

A good knowledge of how to control microorganisms (kill, inhibit or remove) in an environment is important to adequately manage microorganisms in the laboratory.

Standard practice in microbiology is to create a clean workspace prior to commencing microbiological activities. This involves the sterilisation and/or disinfection of equipment, instruments and work surfaces, the use of sterile growth media, and use of practical techniques that will minimise the chance of introducing microbial contaminants into sterile cultures or media.

By using certain physical agents, physical processes or chemical agents, microorganisms can be killed, inhibited or removed.

5.2 Decontamination and sterilisation

5.2.1 Decontamination

Decontamination can be defined as a process that renders an environmental area, device, item, or material safe to handle (i.e., safe in the context of being reasonably free from a risk of disease transmission) and to make (an object or area) safe for unprotected personnel by removing, neutralising, or destroying any harmful substance.[31]

Decontamination in the microbiology laboratory must be carried out with great care. Decontamination may entail sterilisation, disinfection or antisepsis.

Regardless of the method, the purpose of decontamination is to protect the laboratory worker, the environment, and anyone who enters the laboratory or handles laboratory products away from the laboratory.

In laboratory settings, all contaminated equipment or apparatus, spent laboratory materials, and regulated laboratory wastes should be decontaminated before being washed, stored or discarded. This is preferably accomplished by a sterilisation procedure known as steam autoclaving, perhaps the most cost-effective way of decontaminating a device or an item

5.2.2 Sterilisation

Sterilisation is to make free from bacteria or other living microorganisms.[32]

Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms including spore formers and viruses. A sterilisation procedure is one that kills all microorganisms, including highly resistant bacterial endospores. Sterilisation can be accomplished by dry heat (hot air ovens), steam under pressure (autoclaves or pressure cookers), incineration (flaming loops) and radiation (gamma sterilisation of plastic ware).[33] Sterilisation in the school laboratory is commonly achieved using an appropriate pressure cooker or autoclave to heat the items to a temperature of 121°C for 15–20 minutes at 15psi (pounds per square inch of pressure).

Microbiological items that are sterilised by steam sterilisation include:

- molten agar after preparation and prior to pouring into petri dishes
- heat resistant equipment items—forceps, hockey stick spreaders, glass petri dishes, glass bottles that the manufacturer deems suitable for sterilisation

- equipment that has been contaminated by exposure to microbes or during microbiological procedures and prior to washing—forceps, hockey stick spreaders, glass petri dishes, glass bottles that the manufacturer deems suitable for sterilisation
- inoculated or contaminated agar plates broths or slopes prior to disposal
- surplus agar plates broths or slopes
- surplus or redundant stock cultures
- other microbiological waste prior to disposal such as microbial samples, contaminated cotton swabs, contaminated paper towel and items used for spill clean up. Note you should not put anything soaked in chemicals such as 70% alcohol or sodium hypochlorite through an autoclave or pressure cooker.

Sterilisation of these items ideally takes place as soon as possible after completion of a practical activity. Place chemical sterility indicator strips in the centre of every load. Check the strip after the run is complete to ensure temperature and steam conditions have been met.

Items used in microbiology, may require sterilisation 2–3 times or more during the course of preparation for and performance of an activity, e.g. flaming loops.

Further information:

Efficient methods of decontamination and sterilisation in school laboratories are outlined in Science ASSIST:

- *SOP: Operating a pressure cooker and autoclave* (Appendix 1)
- *AIS: Decontaminate microbiological equipment* (Appendix 2)
- *AIS: Microwave, pressure cooker or Autoclave? Recommendations for best practice of sterilising agar* (Appendix 2)

5.3 Disinfectants/antiseptics

5.3.1 Disinfectants

Disinfectants eliminate nearly all recognized pathogenic microorganisms with the exception of bacterial spores on inanimate (non-living) objects.[34] Disinfection is less lethal than sterilisation[35] Use of a disinfectant does not necessarily result in sterility.[36]

In school laboratories, disinfection of benches and instruments should take place **before and after** microbiology procedures as part of aseptic technique. Good laboratory housekeeping dictates all benches should be cleaned regularly and thoroughly to maintain maximum hygiene and reduce the possible spread of infection or chemical contamination. 70% v/v ethanol is the preferred disinfectant for cleaning benches and surfaces not obviously contaminated.[37]

Microorganisms present a range of resistance to chemical disinfectants and no single disinfectant is effective in all situations.[38] Chemical disinfectants suitable for use in standard school experiments and procedures are restricted to substances that are safe for students and staff to use. This eliminates many disinfectants used in clinical or surgical environments. However, by using Risk Group 1 microbes, the risk of users contracting potentially harmful diseases is reduced or eliminated. Standard laboratory hygiene and safety procedures must be used with regard to dissections on animal tissues and use of microorganisms, and no human blood or body fluids should be used.

A site-specific risk assessment must be performed on the disinfection procedure, with consideration for the disinfectant to be used. A manufacturer's safety data sheet should be consulted in the formulation of risk assessments.

Bleach and hospital grade disinfectant can be purchased from a laboratory supplier or supermarket. Script on the front or main label of a container will indicate if a disinfectant is 'hospital grade'. Alcohol, (or its alternatives: methylated spirit, ethanol and isopropyl alcohol) is purchased from cleaning suppliers, hardware stores or laboratory suppliers. Special storage for flammable substances is required.

Check labels of disinfectants to determine the active component and its concentration.

All disinfectants should be prepared according to the manufacturer's instructions. Dilutions should be in fresh water using clean glassware and stored in clean and appropriately labelled storage containers. Fresh disinfectant should be prepared regularly.

5.3.2 Antiseptics

Antiseptics are chemical agents that slow or stop the growth of microorganisms on external surfaces of the body and help prevent infections. Antiseptics should be distinguished from antibiotics that destroy microorganisms inside the body, and from disinfectants, which destroy microorganisms found on inanimate (non-living) objects. Some chemical agents can be used as both an antiseptic and a disinfectant. The purpose for which it is used is determined by its concentration.[39]

In the context of school microbiology, an antiseptic may be used in the decontamination and treatment of human skin after exposure to a microbial culture.

5.3.3 Disinfectants and antiseptics recommended for use in schools

There are multi-purpose chemical agents that are suitable disinfectants and antiseptics for most routine procedures performed in a school science laboratory [Physical Containment 1 (PC1) laboratories, using Risk Group 1 (RG1) bacteria (non-sporing)].

Table 2: Disinfectants and antiseptics suitable for use in Physical Containment 1 (PC1) laboratories, using Risk Group 1 (RG1) bacteria (non-sporing).

Type of disinfectant	Suitability	Limitations	Recommended instructions
70% (v/v) ethanol or isopropyl alcohol	Dissections Microbiology Smooth hard surfaces (i.e. laboratory benches) Gram negative microorganisms Skin Disinfectant	Flammable volatile, high cost. May harden rubber products. Inactive against spores	Dilute to 70% in fresh water. Must be in contact with material being disinfected for at least 10 minutes, longer for heavily soiled items. Industrial methylated spirit (95% ethanol) or isopropyl alcohol appropriately diluted is an acceptable alternative.
Sodium hypochlorite (household liquid bleach)	Disinfecting reusable microbiology equipment after use and single use equipment prior to disposal. Effective against a	Poor stability – prepare immediately prior to use. Affected by temperature, concentration and pH. Bleach solutions are	Contaminated (but not visibly soiled) work surfaces treat with a 0.5–1% v/v (5000–10000 ppm) (available chlorine) solution for at least 10 minutes. General work surfaces

	wide variety of microorganisms	corrosive to metal objects and may damage rubber items. Do not autoclave any materials soaked in bleach solution, as there is a risk a toxic gas may be produced. Bleach is an irritant to users at higher concentrations (skin and mucous membranes). Activity of chlorine bleach is reduced by organic matter[40] Increased concentrations may be required. May bleach and damage clothing. Less effective against spores. Low cost.	may also be routinely cleaned with 0.5–1% v/v (5000–10000 ppm) (available chlorine). Soak microbiology equipment and disposables in 0.5–1% v/v (5000–10000 ppm) (available chlorine) for at least 10 minutes, longer for heavily soiled items. Do not use on metal or rubber items SPILLS: wads of absorbent paper towel soaked in 0.5–1% v/v (available chlorine) hypochlorite solution may be used to absorb and/or wipe down the area affected by a spill. For infectious spills see procedure Section 5.6.2.
Hospital grade disinfectant	Cleaning smooth hard surfaces. Use after general lab procedures, bench and lab cleaning, dissections, spills, cleaning large areas.	Not effective on spore formers.	Dilute according to the manufacturer's instructions in fresh water. Use in a spray or wash bottle to clean benches.
Chlorhexidine	Gram positive, gram negative bacteria and fungi.[41] Skin disinfectant.		0.5–4% (v/v) in ethanol. Wash hands in solution for 2 minutes

5.4 Preparation of sterile and clean resources for microbiological activities in schools

(NB: This section is duplicated in the *AIS: Preparing sterile equipment for microbiology* that is available as a stand-alone document in Appendix 2)

Equipment used in microbiology should be sterile before using. This enables aseptic techniques to be used when transferring microorganisms for inoculation, sampling environmental areas, adding susceptibility discs to agar plates and Gram staining.

This equipment should be prepared before the class activity and stored in clean, lidded containers.

Equipment such as hockey stick spreaders, inoculating loops and sterile swab sticks can be purchased as single-use items from commercial scientific suppliers if the school budget allows or it is more time effective to do so.

Good organisational skills and a disciplined approach ensure that every activity is performed both safely and successfully.[42]

Sterile equipment is prepared well in advance to the planned activity day. Staff preparing items for microbiological activities should carefully study which items such as forceps, hockey stick spreaders and quantities needed before sterilisation can take place. Many schools prepare sterile items and store them in sterile lidded containers until required. Sterile equipment can be stored indefinitely if the packaging is not breached. Sterile agar can be stored indefinitely if it remains in the container it was sterilised in, and the container has not been reopened. Agar plates are best prepared 1–2 days before an activity, and not stored for more than 4–6 weeks. Most schools do not have access to facilities that enable agar plates to be poured in total aseptic conditions. Agar plates purchased commercially may be stored longer if contamination has not occurred.

In-house preparation of sterile items is cost effective to schools as some pieces of equipment can be repeatedly recycled. Care should be taken with ethanol, as it is a flammable substance and should not be used near a naked flame.

Considerations:

- Sterilisation of equipment should be performed in a draught-free area.
- Items to be sterilised should be clean and dry, metal forceps should not be rusty, glass items should not have chips or cracks.
- Consult the planned activity or activities prior to sterilising items to ensure there is the required number of items available during the activity.
- Soaking items in a container of 70% (v/v) ethanol for 10 minutes, disinfects/decontaminates, but does not sterilise items.
- Ensure the bench area for this purpose has been decontaminated with 70% ethanol prior to commencing.
- Aluminium foil or greaseproof paper may be used to wrap items to be sterilised.
- Sterile items can be stored in a large lidded plastic container that has been decontaminated with ethanol and paper towel.
- **Glassware and metal instruments can be wrapped in aluminium foil and sterilised using dry heat in an oven at 160°C for 2–3 hours.[43]**
- **All sterilising processes using an autoclave/steriliser or pressure cooker should be at 121°C for 15–20 minutes at 15psi.**
- Professional microbiologists and higher education providers promote the sterilisation technique of ‘flaming’ hockey stick spreaders and forceps prior to using by dipping in 70% ethanol and igniting it in the Bunsen flame. Incorrect techniques can encourage microbial aerosol transmission and risk the ethanol catching on fire. **Science ASSIST does not recommend this practice in the school setting, but instead recommends sterilising these items in an autoclave or an oven.**

Table 3: Suggested and alternative sterilising techniques		
Item	**Suggested sterilising technique**	**Alternative technique**
Sterile plastic Petri dishes	Purchase sterile, leave wrapped in original packaging until required. (Do not autoclave prior to use. Plates do not retain shape when autoclaved.)	
Sterile glass Petri dishes	Wrap glass Petri dishes in greaseproof paper or aluminium foil and sterilise in an autoclave	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2–3 hours
Nutrient agar plates	Prepare agar solution according to the manufacturer's instructions, autoclave in a heat-safe bottle with lids loose and pour plates when temperature of sterile agar is ~50^0C using aseptic technique. When set, wrap in plastic wrap. Store at 4°C until required. See ASSIST SOP: Preparing agar plates	Purchase prepared and sterile from a biological supplier
Nutrient broth	Prepare broth solution according to the manufacturer's instructions. Aliquot ~15mL into McCartney bottles (28mL capacity) keep lids loose. Autoclave. When cool tighten lids and store at 4°C until required.	Purchase prepared and sterile from a biological supplier
Sterile water	Aliquot 2mL into Bijou bottles (7mL capacity) keep lids loose. Autoclave. When cool tighten lids and store at 4°C until required.	
Sterile plastic dropping pipettes	Purchase single-use pipettes from commercial scientific, biological or medical suppliers.	
Sterile swab stick	Purchase sterile, leave wrapped in original packaging until required.	Autoclave cotton buds in foil covered beaker.
Sterile 'L' spreader	Wrap in aluminium foil and autoclave. Store until required.	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2-3 hours.
Sterile forceps	Wrap in aluminium foil or place inside a clean test tube, cover opening with aluminium foil and autoclave. Store until required.	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2-3 hours.
Sterile test tubes/ conical flasks	Cover opening with foil or plug with non-absorbent cotton wool. Autoclave.	Cover opening with aluminium foil. Sterilise using dry heat in an oven at 160°C for 2–3 hours
Inoculating loop	Flame to red heat in the blue flame of the Bunsen burner.	Purchase sterile disposable inoculating loops, leave wrapped in original packaging until required.

Further information: See *SOP: Operating a pressure cooker and autoclave* (Appendix 1).

5.5 Decontamination of equipment and cultures at the conclusion of microbiological activities

Immediately concluding microbiological practical activities, it is imperative that thorough decontamination of equipment, the laboratory, and the sterilisation of bacterial cultures and agar plates occur. This prevents contamination of future microbiological and non-microbiological practical activities and possible ill effects of students and staff.

Staff and students each have an important role.

Staff responsibility:

- Provide containers, and contaminated waste bags e.g. autoclave bags or oven bags for students to segregate and dispose of equipment and cultures. Each receptacle should be labelled with its function. Ensure these are removed from the laboratory at the end of the activity.
    - Prepare 0.5–1% v/v (5000–10000 ppm) (available chlorine) sodium hypochlorite (bleach) solution in a large (500–1000mL) labelled beaker (1 per student group) for swab sticks. Provide one for each student group.
    - Dilute hospital-grade disinfectant according to the manufacturer's instructions for contaminated items such as forceps. Place diluted disinfectant in a large labelled beaker and provide one for each student group.
    - Provide a solid bin containing autoclave bags or oven bags.
    - Provide a separate autoclave bag for contaminated disposable PPE and reusable PPE.
- Ensure all agar plates and equipment are returned to the designated area.
- Oversee thorough bench decontamination and hand washing of students.

Student responsibility:

- Return all agar plates after examination. Place directly into designated autoclave or oven bags.
- Place all equipment in the designated area.
- Clean benches by swabbing with hospital grade disinfectant or 70% ethanol using disposable paper towel. Allow to air dry.
- Dispose of aprons, gloves and bench cleaning paper into the appropriate contaminated waste autoclave bag.
- If lab coats, aprons or uniforms are contaminated, they will need to be bagged and sterilised before washing and reuse.
- Wash hands before leaving the laboratory or beginning a new activity.

Contaminated waste includes any item that has been exposed to microorganisms in the course of a practical activity. Non-contaminated waste is any item that has not been exposed to any microbes such as freshly prepared un-inoculated agar. If in doubt, non-contaminated waste should be treated as a contaminated item.

5.5.1 Disposal of microbiological waste

All microbiological waste is required to be sterilised before disposal. Microbiological waste consists of inoculated agar plates, broths, used swabs or other items used to sample or manipulate microorganisms. Plastic Petri dishes are commonly made from clear polystyrene plastic which are heat resistant up to 80^{o}C.[44] They are regarded as a use once disposable item and under sterilisation conditions in an autoclave or pressure cooker they will deform.

The best way to sterilise non-liquid biological waste in an autoclave or pressure cooker is by placing them into a bag that will withstand the sterilisation conditions and contain the treated contents. Schools have the option of using two different types of bags:

- Autoclavable biohazard bags, which are available from scientific suppliers, see the Science ASSIST *School science suppliers* list. These are made from a heavy-duty plastic e.g. polypropylene marked with the international biohazard symbol and usually have the word autoclavable written on them. They are available in a variety of sizes.
- Oven bags which can be purchased from supermarkets. These are also available in different sizes.

If you are unsure if your biohazard bags are suitable for autoclaving, we suggest that you contact the supplier for advice.

Procedure for using an autoclavable biohazard or oven bag for sterilising microbiological waste:

- **Loosely pack microbiological waste including agar plates into bags to no more than 2/3 full**. This will ensure that the steam during sterilisation will penetrate the entire load. Bags that are tightly filled to capacity will not allow effective steam penetration and the contents will not be sterilised even if all sterilisation parameters are met.
- **Make sure there are no sharp objects present** that may puncture the bag.
- **Loosely tape shut the bag leaving an opening of about 5–6cm** to allow good steam penetration. This can be done with autoclave tape or a rubber band. Never tightly close the bags as they are impervious to steam and therefore the temperature of the inside of the bag will not be sufficient for sterilisation.
- It is advisable to **place the bag into a secondary container** within the steriliser to prevent any leakage into the steriliser should the bag rupture. The container must be able to withstand the autoclaving conditions.
- **Do not overload the steriliser** with too many bags as this may block steam circulation.
- **Use a sterility compliance strip** to indicate if the correct time, temperature and pressure have been reached during the run time. These are available from scientific suppliers.
- **Sterilise at 15psi, 121°C for 15–20 minutes.**
- After sterilisation has been verified, the autoclave or oven bag containing waste items should be **disposed of by placing it into a sturdy garbage bag which is sealed for immediate disposal in industrial bins.**
- **Wear heat protective gloves** when removing waste from the steriliser.
- **Sterilisation of these items ideally takes place as soon as possible** after completion of a practical activity and occurs within the science laboratory or prep room area.

Liquid cultures in bottles or test tubes can be placed onto a tray in the steriliser with their lids loose. See Science ASSIST *SOP: Operating a pressure cooker and autoclave* in Appendix 1.

5.5.2 Decontaminating equipment and facilities

There are a variety of methods used to decontaminate equipment and facilities used in microbiological activities.

Table 4: Decontamination procedures for equipment and facilities used in school microbiology laboratories.	
Contaminated item	**Suggested decontamination technique**
Inoculated agar plates – plastic	Pack unopened plates loosely in autoclave bag, leaving an opening of about 5–6cm to allow good steam penetration. Autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Inoculated culture broth in McCartney or Bijou bottles.	Make sure that the lids are loose. Autoclave at 121°C, 15psi for 15–20mins. Empty contents in the sink with copious amounts of water. Wash in warm soapy water, rinse well and dry. *Resterilise:* Re-autoclave loosely lidded container. Store in a clean closed container.
Inoculated water in glass bottle	Make sure that the lids are loose. Autoclave at 121°C, 15psi for 15–20mins. Empty contents in sink. Wash in warm water and dry. *Resterilise:* Re-autoclave loosely lidded container. Store in a clean closed container.
Plastic dropping pipettes	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in the general waste.
Used swabs	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours or. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15-20 mins. When cool, place unopened autoclave bag in a garbage bag and dispose in the general waste.
Sterile 'L' spreader	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours, or 2) Place directly into an autoclave resistant container and cover with foil or place into an autoclave/oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15-20 mins. 3) After sterilisation, wash in warm soapy water, rinse and dry. *Resterilise:* Wrap in foil and sterilise in an autoclave or hot air oven. Store until required for re-use.
Sterile forceps	Carefully place into an autoclave resistant container such as a large test tube, cover with foil and autoclave. Wash in warm soapy water, rinse and dry. *Resterilise:* Wrap in foil or place inside a clean test tube, cover opening with foil and autoclave, Store until required for re-use.
Test tubes	Autoclave at 121°C, 15psi for 15–20mins. Empty contents in sink. Wash in warm soapy water, rinse and dry. *Resterilise:* Plug with non-absorbent cotton wool and autoclave or cover the opening of the test tubes with foil and sterilise in a hot air oven or autoclave. Store in a clean closed container.

Contaminated item	Suggested decontamination technique
Inoculating loop	Flame to red heat carefully in the blue flame of the Bunsen burner to prevent the transmission of aerosols. Cool and reuse immediately. Alternatively, if using disposable inoculating loops, 1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours.. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Susceptibility discs Mastrings (set of 6 or more antibiotic discs joined together)	Susceptibility discs and Mastrings should remain on the agar plate after examination. The agar plate remains closed. Pack unopened plates loosely in autoclave bag and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Paper towel exposed to contaminated areas Used disposable aprons/lab coats Used gloves	If not soaked in bleach or alcohol, sterilise in an autoclave or pressure cooker. If soaked leave for the recommended time and then dispose of into the general waste. An autoclave or oven bag should be placed in the laboratory for students to place these waste items directly into the bag. Do not overfill the bag. Leave an opening of about 5–6cm to allow good steam penetration and sterilise for 15–20 min at 121°C and 15psi. Place the unopened autoclave bag into a sturdy garbage bag and seal for immediate disposal in an industrial bin.
Laboratory benches Plastic containers used for storage and distribution of equipment Any other hard surface	Dilute disinfectant in fresh water according to the manufacturer's instructions. Use in a spray bottle. Dilute ethanol to 70% in fresh water, use in a wash bottle. Ethanol is flammable. Apply liberally to laboratory bench or other hard surface to be decontaminated. Wipe lightly with paper towel. Allow the residual to air dry.

5.6 Spill kits

Spill kits should be present in all work places that store and use hazardous materials. In the school microbiology laboratory, this includes hazards such as chemicals and biohazards. The type of spill kit used should reflect the type of hazards present in the laboratory, e.g. a chemical spill kit for a laboratory storing and using chemicals, and/or a biohazard spill kit for a laboratory handling microbiological, human or animal fluids.[45] Spill kits in school microbiology laboratories need to specifically include clean-up substances for:

- microbiology biohazards,
- flammable liquids, and
- chemical cleaning agents.

5.6.1 Biohazard spill kit contents

A microbiology specific spill kit for biohazard materials should include the following items:

Table 5: Items in a microbiology specific spill kit

	Item	Description
PPE	Eye protection	Wrap around type that fit over prescription glasses
	Nitrile gloves	Chemical resistant
	Apron or lab coat	Disposable options available
	Face mask	Useful protection when sweeping dusts/powders and when handling neutralising agent powders. Protection against aerosols.
Other items	Dustpan and brush	For sweeping up spilt powders
	Tongs/forceps	To pick up contaminated materials, e.g. fragments of broken glass containers. Sharps container available for use.
	Absorbent materials (i.e. paper towels)	To soak up small chemical or biohazard spills
	Autoclave or oven bags	To contain absorbed spills and biological waste.
	Signage	For example, “Do not enter: Biohazard spill clean-up in progress”
	Sodium hypochlorite (bleach) solution freshly prepared.	Absorb the treated spill with paper towel and flood with 0.5–1% sodium hypochlorite to sterilise prior to disposal. Also, use bleach to treat exposed surfaces.
Absorbent materials, pads and pillows for chemical spills. Select as appropriate from the following range of options.	Vermiculite	Silicate material that can absorb up to 10 times its weight of water. Good for aqueous spills, less effective for oil based spills.
	Attapulgite (‘kitty litter’)	Silicate (clay) mineral that is an excellent absorption agent. Useful for oil-based spills
	Commercial chemical absorbent pads and pillows	Available in various sizes and shapes-pads, pillows, snakes
	Super-absorbent polymers	Polymers with cross-linking to form gels that can absorb many times their weight of aqueous solutions. The material in disposable diapers
	Sand	Useful to contain a spill

Schools must ensure the availability of current Safety Data Sheets (SDS) prepared by the manufacturer or importer for all hazardous chemicals.

5.6.2 Spill clean-up procedure for biohazards

For minor spills:

1. Wear gloves and protective clothing.
2. Cover the spill with paper towels to contain and absorb the spill.
3. Pour an appropriate disinfectant over the paper towels and the immediate surrounding area (generally, freshly prepared 0.5–1% v/v bleach solution is used). Leave for 30 minutes.
4. After the appropriate amount of contact time the soaked paper towel can be placed into the normal bin.
5. Wash the decontaminated area with water and detergent.
6. All contaminated material should be placed into either an autoclave bag or oven bag to be put through an autoclave or pressure cooker. This may include a lab coat or apron or uniform, if contaminated.
7. Do not autoclave material containing hypochlorite, since chlorine gas can be produced.
8. Wash hands thoroughly with soap and water on completion.

In the event of a large spill of infectious or potentially infectious material, the following spill clean-up procedure should be used.

1. Evacuate and isolate the immediate area, minimise any draughts.
2. Wear gloves and protective clothing, including face or eye protection.
3. Check for sharps from broken glassware. If present, remove with forceps and place into a sharps biohazard waste container. Wrap forceps in foil or place in a clean test tube, cover opening with foil and autoclave.
4. Cover the spill with paper towels to contain and absorb the spill.
5. Pour an appropriate disinfectant over the paper towels and the immediate surrounding area (generally, freshly prepared 0.5–1% v/v bleach solution is used)
6. Apply disinfectant concentrically beginning at the outer margin of the spill area, working toward the centre. Leave for 30 minutes.
7. After the appropriate amount of contact time, the soaked paper towel can be placed into the normal bin.
8. Wash the decontaminated area with water and detergent.
9. All contaminated material should be placed into either an autoclave bag or oven bag to be put through an autoclave or pressure cooker. This may include a lab coat or apron or uniform if contaminated.
10. Do not autoclave material containing hypochlorite, since chlorine gas can be produced.
11. Ensure that all materials are disposed of correctly and seek further advice if necessary.
12. Wash hands thoroughly with soap and water on completion.
13. Report the spill to the head teacher and/or the school Work Health and Safety Officer.
14. Staff and students should not be allowed into the area of the spillage, nor should the notice be removed until all the spilt substance is cleaned up and odours dissipated.

5.6.3 Spill clean-up procedure for chemicals

Major spills should be immediately referred to trained emergency personnel. Remove ignition sources and evacuate the building. It is helpful if the substance has been identified.

Minor spills, depending on the substances, may be treated as follows:

1. Notify laboratory personnel and neighbours of the accident.

2. Evacuate and isolate the immediate area.
3. If flammable liquid has been spilt, remove ignition sources and isolate power to the laboratory with the emergency switch.
4. Establish ventilation – open windows and doors.
5. If possible, positively identify the substance spilt. Check the labelling on the bottle identifying the chemical and its hazards. Look for hazard signal words and hazard statements and if possible, consult the safety data sheet.
6. Locate the spill kit.
7. Choose appropriate PPE (goggles, face shield, chemical resistant gloves, lab coat, apron).
8. Contain the spill using a spill absorbent material such as Vermiculite or sand as a barrier. Note: There are also commercial products available such as chemical absorbent pillows, either in pillow or long snake shapes, which can be used to contain spills.
9. **Acid and base spills** should be neutralized prior to clean up. Acids should be covered with sodium carbonate or sodium bicarbonate until the reaction ceases to fizz. Spilt bases should be neutralised with vinegar, boric acid or sodium bisulphite. Liberally sprinkle the neutraliser over the spill starting at the perimeter and continue towards the centre. Leave for 1–5 minutes.
10. **Flammable spills** should be covered with commercial spill absorbent material, chemical absorbent pads or paper towel to soak up the spill. These items should be then left to completely evaporate in a fume cupboard or a secure area outdoors away from all sources of ignition. Wash chemical absorbent pad or paper towel in water before disposal.
11. Mercury spills: in the event of your school having mercury, including mercury thermometers, it is important to have a prepared strategy for dealing with a potential spill. There are two approaches to this: Commercial Hg spill kits have absorbent sponges that pick up the globules and then stores the Hg in the collecting container when the sponge lid is screwed back on. There is also Hg decontaminant powder, supplied in the lists of chemicals, that reacts with the mercury to prevent the formation of vapour, which can then be collected for disposal.
12. Sweep solid material into a plastic dustpan and place in a sealed container.
13. Wet mop spill area. Be sure to decontaminate broom, dustpan.
14. Put all contaminated items (gloves, clothing, etc.) into a sealed container or plastic bag.
15. Ensure that all materials are disposed of correctly and seek further advice if necessary. Disposal of toxic substances may need to be arranged through a commercial waste disposal company
16. Return spill kit to storage location and arrange for used contents to be replaced.
17. Report the spill to the head teacher and/or the school Work Health and Safety Officer.[46]

Staff and students should not be allowed into the area of the spillage, nor should the notice be removed until all the spilt substance is cleaned up and odours dissipated.

After an incident, immediately replenish stock of spill kit, review procedures and effectiveness of the emergency plan, and document for WH&S reporting as per your school procedure. The references listed below have some very good guidance material.

For 'Removal of gloves technique' see http://education.qld.gov.au/health/pdfs/healthsafety/handsgloves.pdf)

For some school-based resources see:

- Victorian Department of Education and Early Childhood Development (DEECD). *Guidance Sheet 4: Chemical Spill Management,* DEECD website, https://www.eduweb.vic.gov.au/edulibrary/public/ohs/Guidance_Sheet_4_-_Chemical_Spill_Management.pdf (Accessed November 2016)

- ‘Spill Kits’, Western Australia Department of Education. Regional Technicians Group website, http://www.rtg.wa.edu.au/Spill%20kits/spills%20index.htm (Accessed November 2016)

Chapter 6 Media for cultivating microorganisms

Microorganisms grown in the laboratory require a culture medium that contains all the nutritional requirements for reproduction and growth. The main elements for cell growth include water, nitrogen source, carbon source, energy source, sulfur and phosphorus, certain inorganic ions and other growth factors such as vitamins.

6.1 What is culture media?

A culture medium provides the food and imitates the normal habitat for microorganisms and must contain carbon and energy sources and other nutrients. Different microorganisms use these elements in distinct ways to survive and thrive.

Microorganisms are diversified in their nutritional requirements. One type of culture medium will not support the growth of all microorganisms. Different species of microorganisms require different culture media to successfully grow and reproduce. Variations to the type and amount of specific nutrients result in different agar types and the microorganisms that grow on them.

Culture media can be solid (agar set in a petri dish) or liquid (broth). Many microorganisms will grow in either media. For observation purposes in school laboratories, solid culture media such as nutrient agar set in a Petri dish gives the best visual representation of the morphology, reproduction and growth pattern of different microbes without reopening the Petri dish for further examination.

6.2 Suitable media for schools

When selecting microbiological practical activities in schools, teachers and laboratory technicians should consider environmental, risk and staff competency factors.

Australian schools are generally designed and constructed to Physical Containment 1 (PC1) standard. Microbiological practical activities selected should align with facilities and work practices in a PC1 laboratory and use microorganisms only from Risk Group 1 (RG1). The use of culture media that are used specifically to grow microbes from RG2 or higher, must not be used in schools, as they can select for human pathogens (see section 6.4)

Nutrient and plain agar is recommended for most microbiological practical activities suitable for schools. Nutrient agar is a general-purpose, nutrient medium used for the isolation of bacteria and fungi. These agars do not selectively grow pathogenic bacteria and allow safe Risk Group 1 microorganisms to grow. They can also be used to demonstrate sensitivity of microbes to antibiotics and disinfectants.

6.2.1 Nutrient agar

Nutrient agar consists of peptone, beef extract, and agar, which is a complex carbohydrate derived from the extract of seaweed. Agar is an excellent solidifying agent because it liquefies at 100°C and solidifies at 40°C and is indigestible by microbes. Nutrient agar provides amino acids, minerals, and other nutrients used by a wide variety of bacteria for growth.

Ingredients of nutrient agar:

- 0.5% Peptone. Peptone is the principal source of organic nitrogen for the growing of bacteria.
- 0.3% beef extract/yeast extract. This contains water-soluble substances, which aid in bacterial growth, such as vitamins, carbohydrates, organic nitrogen compounds and salts.

- 1.5% agar. The solidifying agent. Agar is firmer and stronger than gelatine. It is still possible, however, to use gelatine as a culture medium for bacteria if agar is unavailable.
- 0.5% Sodium chloride. The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.
- Distilled water. Water is essential for the growth of and reproduction of microorganisms and also provides the medium through which various nutrients can be transported.
- pH is adjusted to neutral (7.4) at 25°C.[47]

Nutrient agar can be made from dehydrated preparations available from scientific suppliers, using individual ingredients, or by purchasing pre-poured agar plates.

6.2.2 Plain or basic agar

Plain or basic agar is a non-nutrient agar used to culture the slime mould *Physarum polycephalum*. It contains a mixture of 1.5% agar and water. Alternatively, pre-poured plain agar plates can be purchased from biological suppliers.

Other culture media used for Level 1 microbiology activities may include:

- Algal medium – for cultivating algae
- Malt extract agar – for fungi and yeasts
- Paramecium culture medium
- Pond water solutions

These contain specific nutrients for the microorganisms and are suitable for use in schools.

6.3 Making plain/nutrient agar plates

Careful planning for microbiological activities includes preparing enough agar plates for the activity. Laboratory technicians preparing plain and nutrient agar plates generally should allow 20 minutes to make media, 45 minutes to autoclave and 60 minutes to cool before pouring plates. Setting time of the agar can range from 5 to 15 minutes.

Prior to pouring agar plates, keep sterile Petri dishes closed until agar is ready to pour into them. Air-born contaminants can easily invade an open Petri dish.[48] It is preferable to use sterile disposable plastic Petri dishes over glass.

Agar plates should be made up as required, and not stored for long periods to avoid any unwanted microbial growth. Agar plates should be disposed of as soon as possible after the activity.[49] All agars for microbiological work need to be sterilised before and after use. Alternatively, pre-poured sterile agar plates can be purchased from reputable biological suppliers.

Prepared agar plates should be stored in a refrigerator (below 4°C). Storage at temperatures higher than 4°C will reduce the shelf life. Do not freeze the plates, as this will denature the nutrient agar medium. Store upside down with the agar on the 'roof' of the plate. This prevents condensation forming on the agar surface and reduces the chance for contamination.[50]

For further information on the preparation of nutrient and plain agar see Science ASSIST *SOP: Preparing agar plates*. (Appendix 1)

For best practice for sterilising agar see Science ASSIST *AIS: Microwave, pressure cooker or autoclave? Recommendations for best practice of sterilising agar*. (Appendix 2)

Also, see Science ASSIST *SOP: Operating a pressure cooker and autoclave*. (Appendix 1)

6.4 Unsuitable media for schools

Agars used in health and industrial laboratories are used to grow specialised microbes under specific conditions. These are not suitable for the school laboratory. Selective, differential and enriched media such as blood, chocolate and MacConkey agars encourage the growth of more fastidious microorganisms, many of which are classed as RG2 and are pathogenic. **These media must not be used in school laboratories**.

Chapter 7 Inoculating agar plates

Inoculating media is a method where live microbes are transferred onto an agar plate. This is an area where contamination of the agar plate can occur. It is important to use aseptic techniques and follow the correct microbiological procedures when inoculating to prevent contamination.

A pure culture is a microbiological culture containing a single species of organism. In schools, this organism should be previously identified. An environmental (sometimes referred to as 'wild') culture contains a microbe that is grown from an unknown source such as those found in environmental sampling. The resulting microbe has not been identified in the laboratory. Remember always regard all microorganisms as potential pathogens, and use aseptic techniques at all times.

Where microorganisms are grown on agar plates from environmental sampling, the plates must not be opened and the microorganisms must not be subcultured. When observations are complete the plates should be sterilised in an autoclave or pressure cooker and then disposed of in the rubbish.

7.1 Inoculation techniques commonly used in schools

Microorganisms can be inoculated onto agar plates in school science laboratories by various methods.

7.1.1 Environmental sampling methods

- **Inoculation of plates via air exposure (settle plates)**
  Sterile Petri dishes are left open to the air in various places in the laboratory for a period of time before lids are replaced, sealed and incubated.
- **Inoculation by direct contact**
  Microorganisms are transferred directly to an agar plate by touching the surface of the agar with an item such as a coin. Touching the agar with your fingertips is not recommended as this could allow for the growth of pathogens.
- **Inoculation of plates with sterile swabs from environmental samples**
  Sterile cotton swabs are used to transfer microbes from an environmental area to an agar plate. Individually packaged sterile swabs may be purchased from a biological or medical supplier, or may be sterilised in an autoclave or pressure cooker prior to use. Place cotton buds in a beaker and seal with alfoil before sterilising.
  Sterile cotton swabs should be kept in the individually sealed packaging until required, and should never be unwrapped and placed on the laboratory bench unless the sampling area is the bench.
  A sterile swab is moistened in sterile water and wiped over the area to be sampled. The swab is then moved over the surface of the agar plate in a zigzag manner to transfer any microorganisms. The swab can be disposed of into the rubbish, as it has not been used to swab areas that contain any pathogens. See Science ASSIST *SOP: Microbes are everywhere* (Appendix 1).

Sample results can be seen in Chapter 9 Observing microbes

7.1.2 Methods using aseptic technique

- **Inoculation of plates with bacteriological loops**
  A heat sterilised loop or disposable inoculating loop can be used to sample from a yoghurt or cheese specimen or a liquid culture containing a RG1 microbe in nutrient broth. The

inoculating loop is used to smear the sample either in a zigzag method over the surface of the agar as described above or placed onto a section of the surface of an agar plate and then streaked out for single colonies. See Science ASSIST *SOP: Streak plate inoculation* (Appendix 1).

- **Inoculation of plates to produce a Lawn culture**
  A liquid culture containing a RG1 microbe in nutrient broth is transferred to a sterile nutrient agar plate. One to two drops of culture are placed on the plate using a sterile dropping pipette, which is then immediately placed in a bleach solution. A sterile L-shaped spreader (Hockey stick) is used to gently spread the culture evenly over the agar creating a consistent 'lawn' of culture. The L-spreader is immediately placed into the bleach solution. See Science ASSIST *SOP: Preparation of a lawn culture* (Appendix 1).

Sample results can be seen in Chapter 9 Observing microbes.

7.2 Environmental sampling

When sampling from the environment, the microorganisms collected are not identified. It is therefore important to sample from environments that are not likely to contain human pathogens.

Suitable environments to obtain samples

- Laboratory bench
- Other fixtures within the laboratory e.g. window sills
- Coins from your pocket
- Pens or pencils
- Door handles
- Computer keyboards

Unsuitable environments to obtain samples

Areas where it is not recommended that samples be obtained as they may contain human pathogens.

- Toilets and toilet areas including floors, hand basins and taps
- Food preparation areas
- Any human or animal body fluids or carcasses
- Skin areas including the finger tips and the mouth
- Animal sources
- Soil samples

7.3 Aseptic technique

Aseptic technique is used in higher level activities to prevent contamination of pure cultures from foreign bacteria inherent in the environment. For example, airborne microorganisms (including fungi), microbes picked up from the lab bench-top or other surfaces, microbes found in dust, as well as microbes found on unsterilised glassware and equipment, etc. may potentially contaminate pure cultures, thus interfering with the lab results. Using proper aseptic technique can greatly minimise or even eliminate the risk of contamination.

Aseptic technique is used to:

- transfer cultures from one medium by inoculating another medium. This is called subculturing.
- prevent contamination of cultures and media from microbes in the environment

- prevent aerosols and drops of microorganisms from being spread in the environment and/or infecting staff and students.

7.3.1 Aseptic practices

- Wash hands with soap and water before and after working with microorganisms.
- Cover any cuts with a waterproof dressing and consider wearing disposable gloves.
- Make sure work surfaces are decontaminated before and after working with microorganisms with 70% v/v ethanol.
- Make sure inoculating instruments (inoculating loops and swabs) are sterilised prior and after use.
- Make sure that inoculating instruments containing microbiological samples are not allowed to touch any surface other than the agar that requires inoculation.
- Flame the mouth of all test tubes or bottles containing sterile water or microbial culture broth both when the cap is removed and before it is replaced.
- Plates should be open for a minimum amount of time to minimise the risk of introducing any contaminants from the air.
- Inoculation should be carried out as quickly as possible to minimise introducing any contaminants.
- Work close to the Bunsen flame as it provides an updraught that carries air away from the workspace, so reducing contamination from the air.
- Have a bacterial spills kit available.

Chapter 8 Incubation and growth of microorganisms

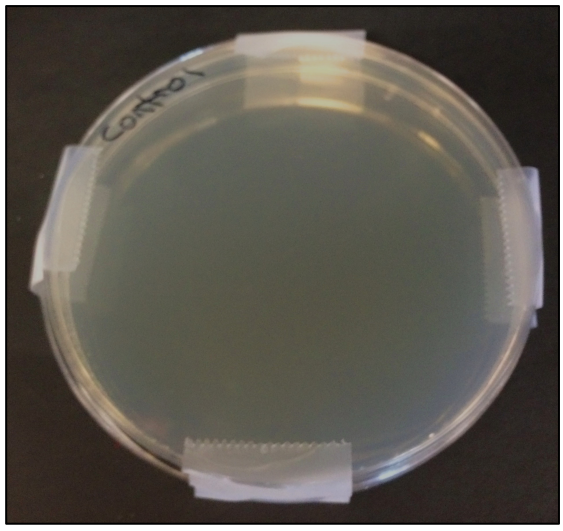
Incubation is the period after the inoculation of an agar plate that microorganisms require for growth and reproduction. Incubation is dependent on certain environmental conditions for optimal growth of different microorganisms.

8.1 Sealing and labelling Petri dishes

After inoculation, agar plates are closed by placing the petri dish lid on the base, and then sealed before incubation. In a school laboratory, sealing the agar plate is conducted to prevent accidental opening of the plate while being incubated under aerobic conditions. Environmental (sometimes referred to as 'wild cultures') agar plates should be kept sealed after inoculation, during incubation and when being examined by students, as the identity and risk group of the environmental microorganisms is unknown.

The recommended method of sealing plates in school laboratories is with four short pieces of sticky tape placed at 12 o'clock, 3 o'clock, 6 o'clock and 9 o'clock to close the two sections of the petri dish. See Figure 1. Complete sealing of the petri dish with sticky tape during growth is not recommended as this creates an anaerobic environment inside the dish that promotes growth of undesirable anaerobic bacteria.[51]

Figure 1. Placement of sticky tape at intervals around the agar plate



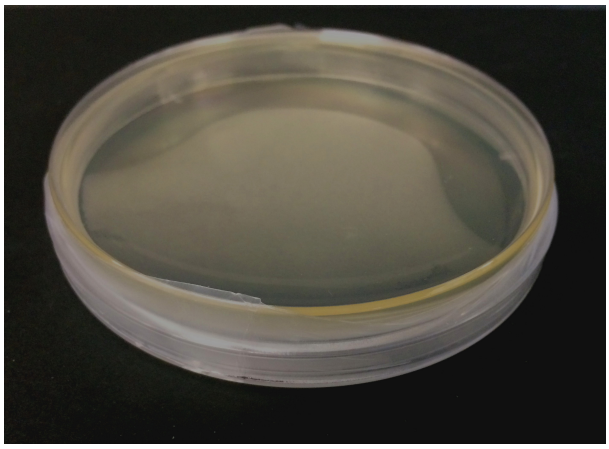
Alternatively, one piece of laboratory sealing film e.g. Parafilm M that is cut no wider than 1cm may be wrapped once around the circumference of the agar plate. The laboratory sealing film should be cut to a length that, when stretched around the agar plate, does not overlap at or beyond its join. See Figure 2. This method prevents leaks of excess fluid from condensation that are potential sources of infection that may come in contact with students or contaminate laboratory areas.

The gas permeability data for Parafilm M indicates that when used as a single layer it will allow sufficient oxygen exchange to promote the growth of aerobic microorganisms and inhibit the growth of potential anaerobes. Wrapping Parafilm around a Petri dish more than once should be avoided and will be sufficient to stop any gas exchange creating an undesirable anaerobic environment.

Parafilm is a laboratory sealing film with unique properties. It is a stretchy, waxy film that is very good at moulding around tops of test tubes, bottles, and flasks and around petri dishes to provide a leak proof seal. It is much more effective than sticky tape.

Each of these methods allows for gas exchange in and out of the agar plate for the growth of aerobic microorganisms.

Figure 2. Placement of laboratory sealing film



Petri dishes should always be labelled on the base containing the media so that if the lid is inadvertently changed the identification of the culture is still known. Information should be written around the perimeter of the plate to avoid covering the colonial growth.

These agar plates are now ready for incubation.

8.2 Environmental conditions required for growth

All living things require certain environmental conditions in which to grow and reproduce. Microorganisms will grow and reproduce in the laboratory when their optimal conditions are met. To promote the growth of Risk Group 1 microbes, certain growth conditions should be met.

In a school science laboratory, there are four main conditions that influence the physical environment for growing Risk Group 1 microorganisms

- Temperature
- Gaseous requirements
- pH
- Osmotic pressure

8.2.1 Temperature

The optimal temperature for growth of microorganisms varies between different species. In laboratory testing of human diseases, incubation temperatures are generally set at 37°C to replicate the temperature of the human body. Microbes grown at these temperatures are generally unknown until further testing has identified them. These microbes may be pathogenic to students and staff.

In the school laboratory, to reduce the growth of pathogens, lower incubation temperatures are used. Agar plates should be incubated between **room temperature (22–25°C) and a maximum of 30°C.** Many cultures suitable for use in schools will grow at room temperature and can be incubated satisfactorily in a cupboard.[52] Refer to Chapter 8.3 Growth of Micro-organisms and time required.

Once grown the cultures may be stored at 4°C. This will slow the growth of any cultures so you can show students a 2–3 day growth if lessons are a week apart.

An incubator may also be used to incubate agar plates as they maintain a constant temperature. Incubators are available from scientific suppliers.

8.2.2 Gaseous atmosphere

An organism that requires oxygen for growth is called 'aerobic'. An organism whose growth cannot occur in the presence of oxygen is 'anaerobic'. An organism that can grow under either aerobic or anaerobic conditions is a facultative anaerobe.[53] In humans, anaerobic bacteria are most commonly found in the gastrointestinal tract and may be pathogenic. The growth of anaerobes and facultative anaerobes should be avoided in schools because of their potential to cause disease in students and staff. They are not classified as Risk Group 1 microorganisms.

Incubating in aerobic conditions, and below human body temperature, reduces the risk of encouraging microorganisms (particularly bacteria) that could be pathogenic to humans.[54] To ensure this occurs, the following practices should be followed:

- Environmental samples should be introduced gently across the surface of the agar with a swab stick, and not penetrate under the agar surface.
- Agar plates should not be sealed in a manner that reduces the exchange of gases in and out of the agar plate.
- Agar plates should not be placed in an airtight container.

8.2.3 pH

The optimum pH for growth varies with different microorganisms. Buffers are usually added to media to prevent sudden pH shifts. Optimal pH conditions for different microorganisms include:

- Bacteria pH 6–8
- Moulds/yeasts pH 5–6
- Protozoa pH 6.7–7.7
- Algae pH 4–8

8.2.4 Osmotic pressure

This generally depends on the concentration of dissolved salt. If the salt concentration outside a cell is too high water is lost from the cell and cell growth is inhibited. Most microbes prefer isotonic solutions where the salt concentration inside the cell is the same as the salt concentration in its environment.

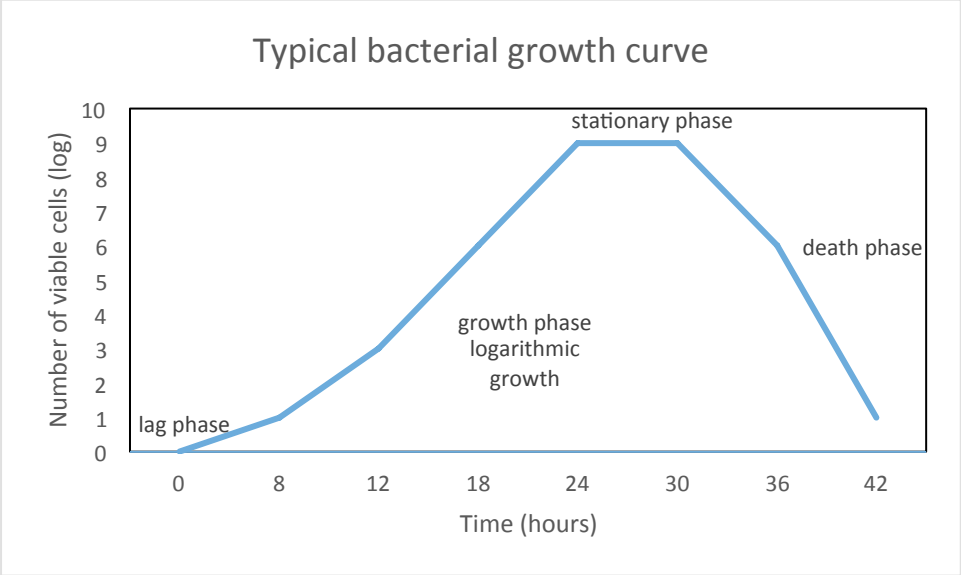
8.3 Growth of microorganisms and time required

When microorganisms are inoculated onto a medium and incubated with optimum conditions for growth, a large increase in the number of cells occurs within a relatively short time.

Optimal time for growing environmental microbes on nutrient agar should be kept to **no greater than 30–36 hours**. After this time, growth and reproduction of microbes slows. Most bacteria multiply by a process called binary fission, some reproduce by budding and some produce spores that develop into a new organism. The following bacterial growth chart shows the typical growth rate of bacteria.

Figure 3: The bacterial growth curve

(Adapted from Todar's Online Textbook of Bacteriology http://textbookofbacteriology.net/growth_3.html)



1. **Lag phase**. Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, the cells are adjusting to their new conditions.
2. **Growth or log phase**. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. This is the most rapid (exponential) growth phase.
3. **Stationary phase** Microbe population growth is limited by one of three factors: 1. Exhaustion of available nutrients; 2. Accumulation of inhibitory metabolites or end products; 3. Exhaustion of space. Cell division slows down and the cell numbers stabilise.
4. **Death phase.** If incubation continues after the population reaches the stationary phase, a death phase follows, in which the viable cell population declines.

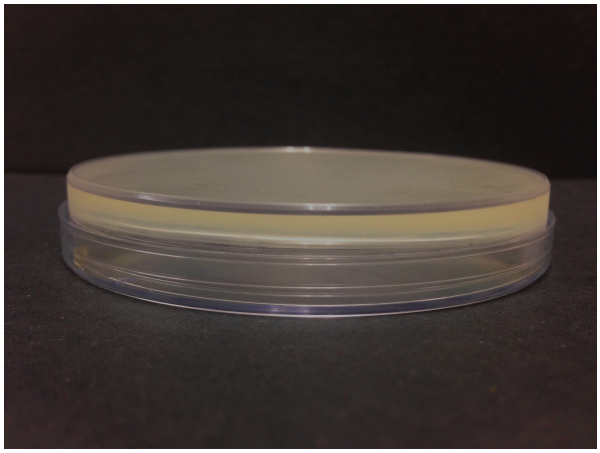
Time of growth can vary between different bacterial species, and different fungal species. Whilst the growth curve above refers to bacteria, the growth of fungi will differ in that a single unit of fungus will grow rapidly in the beginning. The exponential phase will be followed by a plateau, (commonly called the stationary phase) and as the organic nutrients become depleted is followed by a decline phase. These stages are typical of any organism growing in fixed quantities of nutrients, particularly under laboratory conditions.[55] In school laboratories it is not beneficial to incubate microbial samples beyond 36 hours, as available nutrients are exhausted after this time. However, this is also dependent upon the temperature particularly if they are incubated in a cupboard and subject to the room's ambient temperature. (Consider, for example, the difference in ambient temperature between Cairns and Hobart.)

Slime moulds are an exception if a skilled teacher or laboratory technician continues to supplement its growth with oat feeding and maintaining control of the mould by subculturing. See Science ASSIST *SOP: Physarum polycephalum care and use* (Appendix 1).

8.4 Practical considerations for incubation

- Agar plates must be incubated inverted with the medium-containing half (base) of the Petri dish uppermost otherwise condensation will occur on the lid and drip onto the culture. This might cause colonies to spread into each other and increase the risk of spillage of the contaminated liquid. See Figure 4.

Figure 4: Incubating an agar plate with the media uppermost in the Petri dish



- Count the plates out and in again to ensure that all plates are collected at the end of a lesson.
- Plates should be sealed around the whole circumference before viewing to reduce the risk of students opening the plates.

Chapter 9 Observing microbes

Agar plates that have been inoculated and incubated can be stored at 4°C for 4–5 days, or distributed to the class from the incubation area. Storage at 4°C slows growth of microbes and reduces possible bad odours from microbe growth on the agar plate. Plates should be removed from the refrigerator at least 1 hour prior to class to bring them to room temperature to lessen the likelihood of condensation forming inside the plate, making observation of the microbes difficult.

Plates should remain sealed with tape or laboratory-sealing film when distributed to students, and should remain sealed throughout examination. Students and staff should wear PPE (gowns, gloves, safety glasses), and magnifying glasses and plastic rulers should be provided. This equipment should be wiped with disinfectant before and after use.

Place an autoclave bag or oven bag close by to collect plates that have been examined. The plates should be sterilised following examination and before disposal in an autoclave or pressure cooker.

Students and staff should disinfect bench tops and wash hands thoroughly after handling agar plates, prior to progressing to another activity or leaving the laboratory.

9.1 Macroscopic morphology of bacteria and fungi

The term ‘colony morphology’ refers to the visible macroscopic characteristics of a colony. Colonies of different types of bacteria and fungi can be varied in appearance. When sampling from the environment there will likely be many different types of microbes on an agar plate, this is called a mixed culture. Being able to visibly differentiate microbes based on the appearance of their colonies growing on an agar plate is an essential first step in starting the identification process.

Colonies that differ in appearance are typically different bacterial and fungal strains, species, or genera. However, colony morphology is not a reliable way to identify microbes, as many different types of bacteria and fungi have similar colony morphology.[56]

9.1.1 Describing colony morphology

Bacteria. Each distinct single colony represents an individual bacterial cell that has divided repeatedly. Most bacterial colonies appear white, cream or yellow in colour.

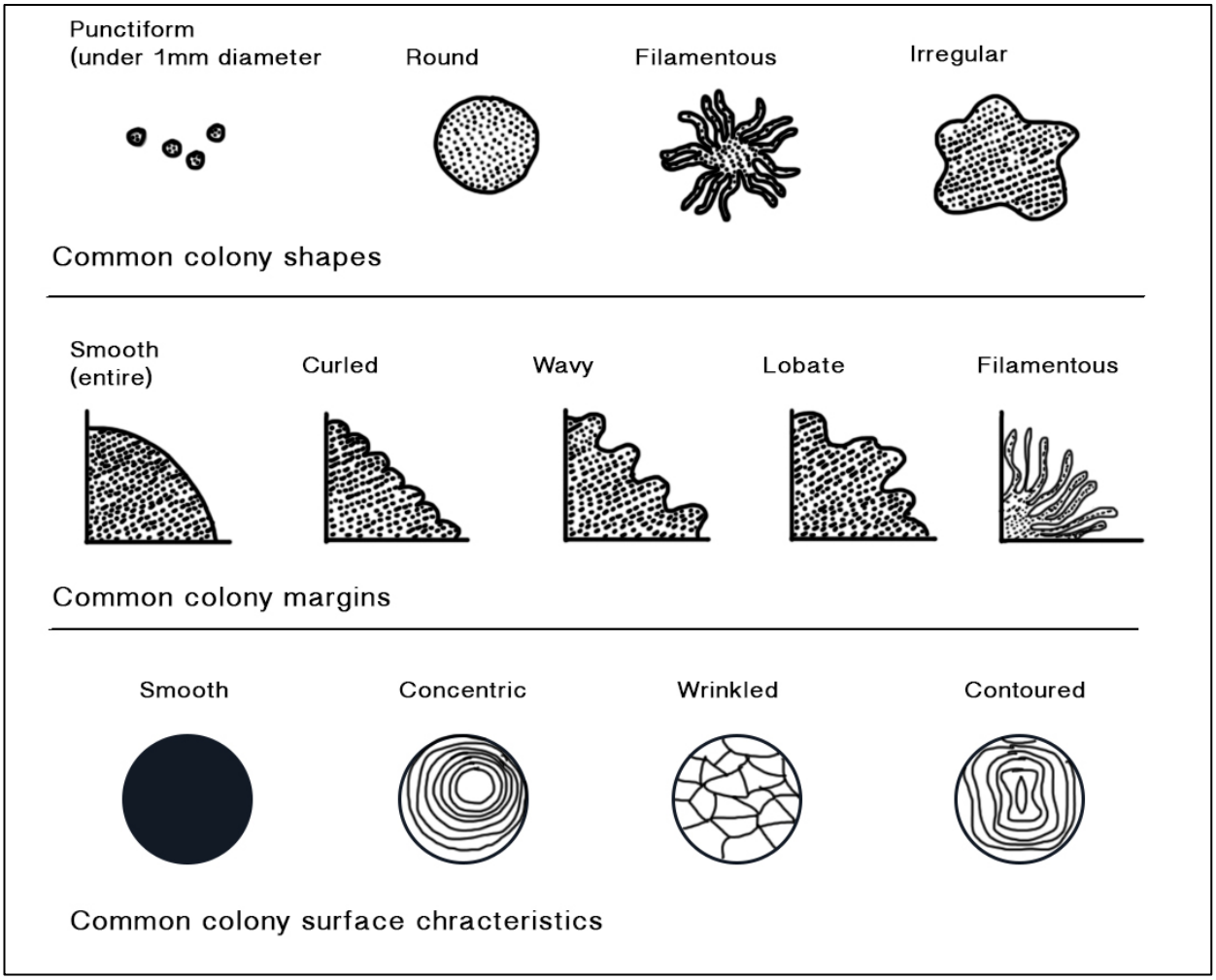
Fungus. Fungal colonies can be divided into yeasts, moulds and dimorphic fungi. **Yeast** colonies can look similar to bacterial colonies. **Moulds** often appear furry and have fuzzy edges. Many turn into a different colour, from the centre outwards. They are identified by the presence of tubular structures known as hyphae. These are masses of individual filaments and look like fuzzy or furry growths. **Dimorphic fungi** are fungi that exist as either yeasts or moulds.[57]

Although bacterial colonies can differ in the details of their appearance, a colony basically looks like a dot growing on the medium. This dot is composed of millions of bacteria. Description of a colony's morphology includes its shape, the margins or edges of the colony, its colour, opacity and surface features. Some colonies are round and smooth; others can have wavy edges and a wrinkled appearance. The morphology of yeast colonies is very similar to bacterial colonies.[58]

Although bacterial and fungal colonies have many characteristics and some can be specific to a species, there are a few basic characteristics that you can identify for all colonies.

- Form: What is the basic shape of the colony? For example, circular, filamentous, etc.
- Size: The diameter of the colony
- Elevation: What is the cross-sectional shape of the colony? Turn the Petri dish on end.
- Margin: What is the magnified shape of the edge of the colony?
- Surface: How does the surface of the colony appear? For example, smooth, glistening, rough, dull (opposite of glistening), rugose (wrinkled), etc.
- Opacity: For example, transparent (clear), opaque, translucent (almost clear, but distorted vision, like looking through frosted glass), iridescent (changing colours in reflected light), etc.
- Colour (pigmentation): For example, white, buff, red, purple, etc. [59]

Figure 5: Colony morphology for bacteria and fungi



9.2 Examining environmental plates

For the method see Science ASSIST *SOP: Microbes are everywhere* (Appendix 1)

9.2.1 Method for examining environmental plates

Provide magnifying glasses and plastic rulers to assist students to examine the colonies. Plates can also be placed under a dissecting microscope for examination.

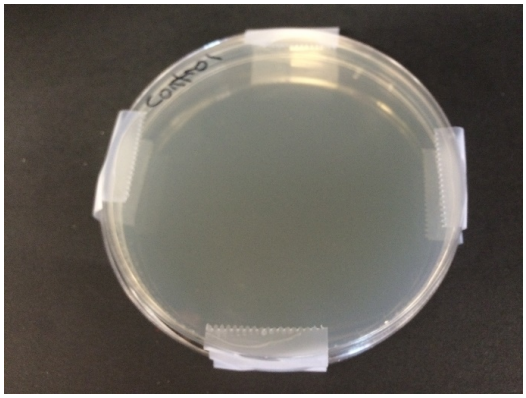
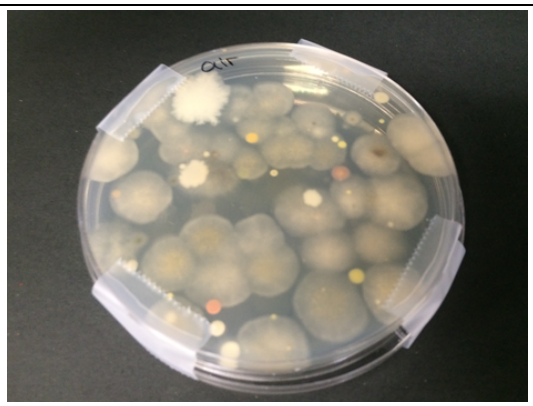
Instructions for students:

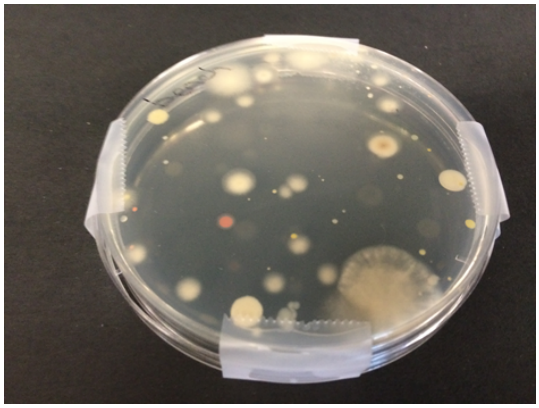
1. Keeping the Petri dish closed, measure the colony diameter in millimetres.
2. Describe the pigmentation (distinguishing between pigmented colonies and those secreting diffusible pigments).

3. Describe the form, elevation, and margin as indicated in Figure 5. Also, indicate whether the colonies are smooth (shiny glistening surface), rough (dull, bumpy, granular, or matte surface), or mucoid (slimy or gummy appearance).
4. Record the opacity of the colonies (transparent, translucent, or opaque).[60]

9.2.2 Example of results of environmental sampling plates

Table 6: Example of results of environmental sampling plates

Plate Number	Sample	Photo and description
1	Control	 No growth
2	Air sample	 Mixed culture. (Not a pure culture). Colonies of bacteria, yeast and fungi grown on nutrient agar. Size of the different colonies varies from approximately 1mm to 12mm in diameter. Pigmentation varies from white, yellow and pink. Most colonies are smooth and are circular in form with an entire margin. There are some irregular shaped colonies with filamentous margins. They have a convex elevation. Most colonies have a smooth surface. There are some colonies with a rough surface. All colonies are opaque in appearance.

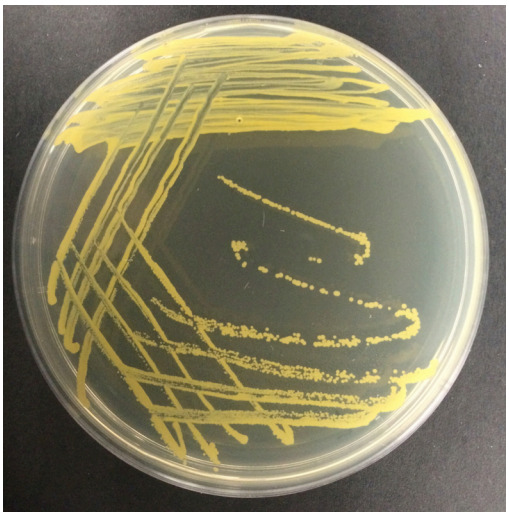
3	Laboratory bench	 Mixed culture (Not a pure culture). Colonies of bacteria, yeast and fungi grown on nutrient agar. Size of the different colonies varies from approximately 1mm to 12mm in diameter. Pigmentation varies from white and yellow. Most colonies are smooth and are circular in form with an entire margin. There are some irregular shaped colonies with filamentous margins. They have a convex elevation. Most colonies have a smooth surface. There are some colonies with a rough surface. All colonies are opaque in appearance.

9.3 Example of results of growth of pure cultures from aseptic inoculation

For methods of inoculating plates with aseptic technique see Chapter 7 Inoculating media, Science ASSIST *SOP: Streak plate inoculation*, *SOP: Preparing a bacterial lawn* (Appendix 1).

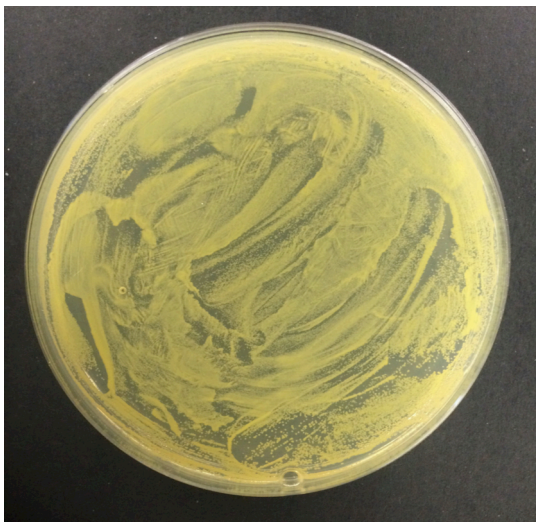
Streak plate inoculation

Figure 6: Streak plate inoculation of *Micrococcus luteus* on nutrient agar, incubated for 24 hours at 28°C.



Lawn inoculation

Figure 7: Lawn inoculation of *Micrococcus luteus* on nutrient agar, incubated for 24 hours at 28°C.



9.4 Examination of *Physarum polycephalum*

See Science ASSIST *SOP: Physarum polycephalum care and use* (Appendix 1) for instructions on how to demonstrate this in class.

Physarum polycephalum is a slime mould that grows in dark humid conditions under the bark of decaying trees and amongst leaf litter on the forest floor. *Physarum polycephalum* evolves through several life stages. The plasmodium stage is the stage most likely to be present on the agar plate when fed and housed under optimal conditions. This is the stage that is of most interest to students, as subculturing can take place, and examination and experimentation can occur. The simplest activity in the laboratory is the demonstration of cytoplasmic streaming.

For further information on the life cycle of *Physarum polycephalum* see 'Physarum life cycle', Southern Biological website, http://file.southernbiological.com/Assets/Products/Specimens/Living_Specimens_and_Supplies/Plants_and_Fungi/L2_30-Physarum_slime_mould_culture/L2_30_Physarum_LifeCycle.pdf (Accessed December 2016)

9.5 Determining the zone of inhibition on antibiotic and disinfectant susceptibility plates using the disc diffusion method

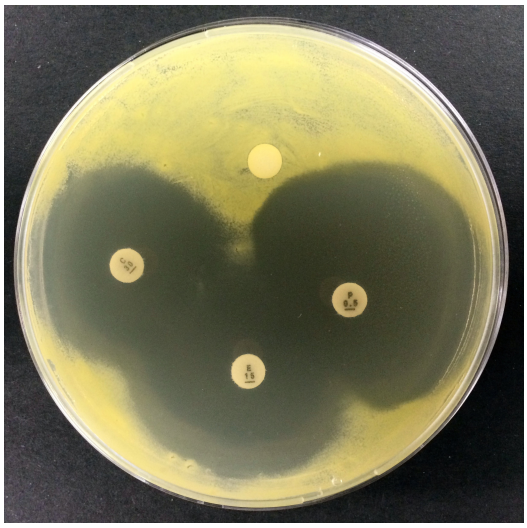
For methods of inoculating plates and introducing discs see Science ASSIST *SOP: Preparing a bacterial lawn* and Science ASSIST *SOP: Susceptibility testing of antiseptics and disinfectants* (Appendix 1)

The susceptibility or sensitivity of a microorganism to antibiotics or disinfectants is a common technique used in pathology, food technology and industrial laboratories. In these tests an antibiotic or disinfectant is incorporated into a paper disc, which is placed on an agar plate containing a lawn culture of the organism to be tested. The antibiotic or disinfectant diffuses into the agar. Sensitivity or resistance is determined by observing zones of inhibition around the discs.

If the organism is sensitive (susceptible) to the agent, then a zone of inhibition (clearing) is observed around the disc. Absence of a zone of inhibition is indicative of resistance. The

effectiveness of the antibiotic or disinfectant is determined by the size of this zone (See Figure 8).

Figure 8: Sample susceptibility test result. *Micrococcus luteus* grown on nutrient agar, incubated for 24 hours at 28°C.
Clockwise from top: control disc, Penicillin 0.5µg, Erythromycin 15µg, Chloramphenicol 30µg.



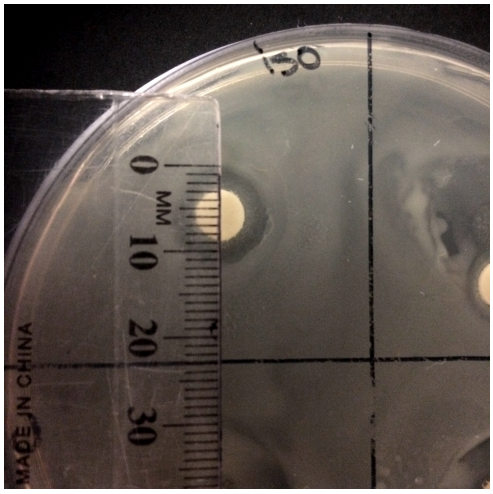
The bigger the area of microorganism-free agar around the disc means the bacteria are more sensitive to the disinfectant. It is expected that there will be no clear area around the control disc, and a larger area around the discs that have been impregnated with an effective disinfectant. This clear area indicates the effectiveness of the disinfectant at killing the microorganism.

9.5.1 Method of measurement

Zones of inhibition are measured using a millimetre ruler. Professional laboratories may have an automated zone reader.

1. Place the agar plate flat on a workbench with the agar side upper most.
2. Place a ruler on the plate with the zero at the edge of microbial growth surrounding one disc.
3. Measure the clear area across the clear zone, including the disc. Record this measurement in millimetres.
4. Repeat for each individual disc.

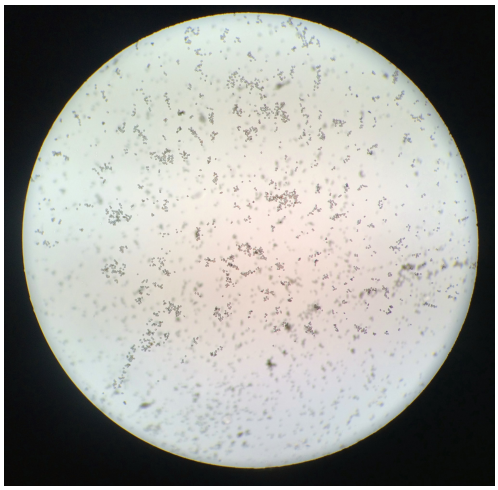
Figure 9: Measuring the zone of inhibition of iso-Propyl alcohol on *Escherichia coli* K–12 grown on a nutrient agar plate, incubated for 24 hours at 28°C.



9.6 Examining mushroom spores

For methods of collecting mushroom spores see Science ASSIST *SOP: Examine mushroom spores* (Appendix 1).

Figure 10: Mushroom spores collected from a Portobello mushroom and examined at 400x magnification.



9.7 Examining pond water

For method of preparing pond water slides see Science ASSIST *SOP: Examining life in pond water* (Appendix 1).

Further information and identification keys can be found at:

- 'The Aquatic Microfauna', Australian Waterlife website, http://australianwaterlife.com.au/microfauna.html (Accessed December 2016)
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9.8 Microscopic examination of a Gram stain

For method of gram staining see Science ASSIST *SOP: Gram stain of a microbial culture smear* (Appendix 1).

The Gram stain is one of the most important stains in the science of microbiology. It divides bacteria into two broad groups. The reaction of bacteria to the Gram stain is a reflection of differences in bacterial cell walls.

- Gram positive – those that retain the primary basic dye and stain purple.
- Gram negative – those that lose the primary dye and stain red with the counterstain.

Bacteria that have been Gram stained are observed microscopically using an oil immersion objective at 1000x magnification. Bacterial cells will appear very small, even at this magnification. Care must be exercised to ensure that artefacts such as dirt on the slide, air bubbles in the immersion oil, undissolved salts contained with the stain and lint from lens tissue are not confused with the microorganism being observed. For additional information on the use of the light microscope see Science ASSIST *SOP: Use and care of the compound light microscope* (Appendix 1).

The expected microscopic morphology and Gram stain result of some Risk Group 1 bacteria is set out in Table 7.

Table 7: Expected Gram stain results

Microorganism	Gram stain	Shape	Description	Diagram (enlarged representation)
Bacillus subtilis	positive	Bacillus Rod shaped	4–10µm long and ~0.25–1µm diameter Singles or in chains	
Escherichia coli	negative	Bacillus Rod shaped	~ 2µm long and ~0.25–1µm diameter Single cells	
Micrococcus luteus	positive	Coccus Spherical	irregular clusters, tetrads or pairs 1.0–1.8µm diameter	 Cocci in pairs  Cocci in tetrads  Cocci in clusters
Staphylococcus epidermidis	positive	Coccus Spherical	0.5–1.5µm diameter cocci usually form in clusters	 Cocci in clusters

Gram stain results are used to plan further investigations to identify bacteria. In professional labs, a suite of tests is carried out to ensure the bacterial sample is correctly identified.

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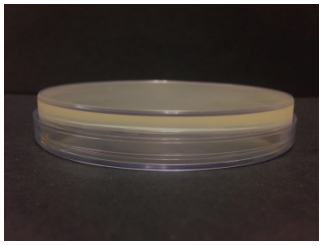
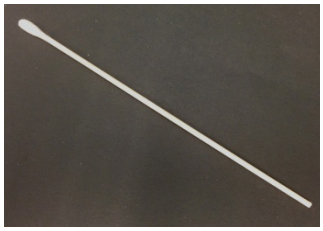
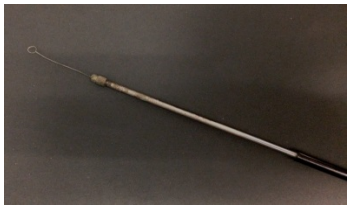
Glossary of microbiology

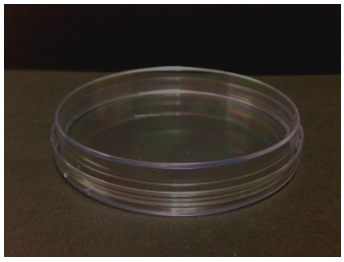
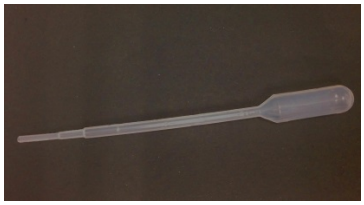
Term	Definition/use
aerobic	Growth in the presence of oxygen or requiring oxygen to live
agar media	Consists of a mixture of protein digests (peptone, tryptone) and inorganic salts, hardened by the addition of 1.5% agar, which supports the growth of a wide variety of bacteria
agar plate 	A petri dish containing an agar growth media
anaerobic	An organism not requiring oxygen, or living in the absence of air or free oxygen
antibiotic	A substance such as penicillin that is capable of destroying or weakening certain microorganisms, especially bacteria or fungi, that cause infections or infectious diseases
antiseptic	A substance that inhibits the proliferation of infectious microorganisms on living tissue.
aseptic	Free of microorganisms that cause disease, free from living pathogenic organisms, sterile
autoclave	An apparatus in which steam under pressure effects sterilisation.
autoclave bag 	Commercially available polypropylene bags marked "Autoclave" designed to withstand heat and pressure of autoclave use. Alternatively, an oven bag may be used
bacteria	Microorganisms made up of a single cell that have a cell wall & no distinct nucleus.
Bijou bottle 	A small glass screw-capped bottle of 7mL capacity, used for liquid cultures

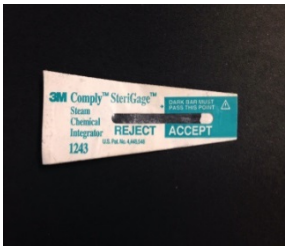
Term	Definition/use
bleach (Sodium hypochlorite)	Sodium hypochlorite is a solid white powder, but is more commonly used or purchased dissolved in water. It has antimicrobial activity, as it can react with proteins and DNA of bacteria, as well as breaking down their cell membranes.
broth media 	A nutrient-infused liquid medium used for growing bacteria. Nutrient broth consists of the same ingredients as Nutrient agar, but without agar as a setting agent
chemotaxis	The movement of a microorganism or cell in response to a chemical stimulus
chromogenesis	A bacterium that produces a pigment
colony morphology	A macroscopic description of cells grown on an agar plate. Descriptors include size, surface pigmentation, opacity, and form, elevation and margin taken from an individual colony.
conidia	an asexual spore formed at the tip of a specialized hypha (conidiophore) in fungi such as *Penicillium*
conidiophores	a specialized fungal hypha that produces conidia
contaminated waste bag 	A heavy-duty polyethylene waste bag that is marked with “contaminated waste” for easy identification ensuring correct disposal of waste. Not suitable for use in pressure cookers or autoclaves
contamination	The presence of extraneous material or microorganisms that renders a substance or preparation impure or harmful
cotton swab stick 	A 150mm long wooden applicator stick with a bud of rayon or cotton wool wound around one end. May be purchased sterile and individually wrapped
culture	A growth of microorganisms, viruses, or tissue cells in a specially prepared nutrient medium under supervised conditions

Term	Definition/use
cytoplasmic streaming	The movement of the fluid substance (cytoplasm) within a plant or animal cell. The motion transports nutrients, proteins, and organelles within cells.
decontamination	To make an object or area safe for unprotected personnel by removing, neutralizing, or destroying any harmful substance
dimorphic fungi	Fungi which can exist as either a mould or yeast
disinfect/disinfection	To cleanse something so as to destroy or prevent the growth of disease-carrying microorganisms
disinfectant	Any agent (such as heat, radiation or a chemical) used chiefly on inanimate objects to destroy or inhibit the growth of harmful organisms
endospore	An inactive form that certain bacteria assume under extreme conditions to protect it from damage. Bacteria have been known to remain dormant but alive in the form of endospores for long periods of time
environmental microbe	A microbe that is grown from an unknown source such as those found in environmental sampling. A microbe that has not been identified in the laboratory. Also referred to as a wild microbe
environmental swab	A series of swabs taken from everyday areas in our environment, such as from laboratory benches and door handles, and inoculated onto nutrient agar plates for incubation and examination.
eukaryotic	An organism whose cells contain a nucleus
filamentous	Contains long slender cells or a series of attached cells, as in some algae and fungi
forceps 	An instrument resembling a pair of pincers, used for grasping, manipulating, or extracting. Forceps are generally constructed out of metal, which can be sterilised by autoclaving.
fungi	Any of a wide variety of organisms that reproduce by spores, including the mushrooms, moulds, yeasts and mildews. The spores of most fungi grow a network of slender tubes called hyphae that spread into and feed off dead organic matter or living organisms
Gram stain	A differential staining technique used to classify bacteria into two groups. A bacterial specimen is first stained with crystal violet, then treated with an iodine solution, decolourized with alcohol, and counterstained with safranin. Because of differences in cell wall structure, gram-positive bacteria retain the violet stain whereas gram-negative bacteria do not.
hospital grade disinfectant	Hospital grade disinfectants kill microorganisms, but not necessarily their spores, and should be used on hard, inanimate, non-porous surfaces and semi-critical (contact

Term	Definition/use
	mucus membranes) and non-critical (contact intact skin, environmental surfaces) objects. Hospital grade disinfectants with specific biocidal claims (virucidal, sporicidal, tuberculocidal, fungicidal or other) must be registered on the Australian Register of Therapeutic Goods.
immunocompromised	Having an impaired immune system and therefore incapable of an effective immune response, usually as a result of disease that damages the immune system
incubate	To maintain at a specific favourable temperature and in other conditions promoting development, as cultures of bacteria. The act of keeping an organism, a cell, or cell culture in conditions favourable for growth and development
incubator 	An apparatus in which media inoculated with microorganisms are cultivated at a constant temperature
infection	Invasion of the body by pathogenic microorganisms
infectious	Communicable by infection, as from one person to another
inoculate	To introduce microorganisms into surroundings suited to their growth, as a culture medium
inoculating loop 	A tool usually made of platinum or nichrome wire in which the tip forms a small loop with a diameter of about 5mm, and is used to smear, streak or take an inoculum from a culture of microorganisms
inoculation spreader 	A glass or disposable plastic rod with a bend to look like a hockey stick. Also known as an 'L' shaped spreader or hockey stick spreader. Used in microbiology to create a lawn culture, by spreading liquid culture evenly over an agar plate.
isotonic	A solution containing the same salt concentration as mammalian blood.
margin	Margin or edge describes the borders of a colony

Term	Definition/use
McCartney bottle 	A glass screw-capped bottle of 28mL capacity, used for solid and liquid cultures
media - growth, culture	A substance, such as agar, in which bacteria or other microorganisms are grown for scientific purposes
microbial aerosol	A fine mist of water droplets containing microorganisms
microbiology	The branch of biology dealing with the structure, function, uses, and modes of existence of microscopic organisms
microorganism	Any organism too small to be viewed by the unaided eye, as bacteria, protozoa, and some fungi and algae
molten agar	Molten agar is agar that is heated to a temperature of above 55°C, where it is in a liquid form. The agar sets at a temperature of 40°C
mordant	Used in staining bacteria. A substance that combines with a stain to fix colour in a material.
morphology	The branch of biology dealing with the size, shape and structure of organisms without consideration of function
mould	Any of various fungi that often form a fuzzy growth (called a mycelium) on the surface of organic matter. Some moulds cause food to spoil, but others are beneficial, such as those used to make certain cheeses and those from which antibiotics like penicillin are developed
nutrient agar	A simple medium that consists of a specific concentration of protein (beef extract, Peptone yeast extract) and Sodium chloride, hardened by the addition of 1.5% agar, which supports the growth of a wide variety of bacteria. Not all bacteria can grow on nutrient agar, some find it too rich and others find it deficient. Nutrient agar does not selectively grow pathogenic bacteria
organism	An individual form of life that is capable of growing, metabolizing nutrients, and usually reproducing
parasitic	Caused by a parasite, which is an organism that lives on or in an organism of another species, known as the host, from the body of which it obtains nutriment
pathogen	Any disease-producing agent or microorganism.
pathogenic	Capable of producing disease
pathogenicity	The disease-causing capacity of a pathogen

Term	Definition/use
Petri dish 	A shallow, circular, glass or plastic dish with a loose-fitting cover over the top and sides, used for culturing bacteria and other microorganisms
phototaxis	Response by movement of an organism toward or away from a source of light, response
Physarum polycephalum	*Physarum polycephalum* is a slime mould that inhabits shady, cool, moist areas, such as decaying leaves and logs. It is typically yellow in colour, and eats fungal spores, bacteria, and other microbes.
plain agar	Non-nutrient agar consisting of agar (as a setting agent) and water. Suitable for culture and growth of Physarum polycephalum
plasmodium	A mass of protoplasm having many cell nuclei but not divided into separate cells it is formed by the combination of many amoeba-like cells and is characteristic of the active, feeding phase of certain slime moulds
plastic dropping pipette 	Plastic transfer pipette. 1mL size is ideal for microbiology
PPE	Personal protective equipment: clothing and equipment used to ensure personal safety in the workplace
pressure cooker	A reinforced pot, usually of steel or aluminium which heats to above boiling point by steam maintained under pressure
prokaryotic	Any of a wide variety of one-celled organisms that lack a distinct cell nucleus and their DNA is not organised into chromosomes. They also lack the internal structures bound by membranes called organelles, such as mitochondria
protozoa	Any of a large group of one-celled organisms (called protists) that live in water or as parasites. Many protozoans move about by means of appendages known as cilia or flagella. Protozoans include the amoebas, flagellates, foraminiferans, and ciliates
pure culture	A laboratory microbiological culture containing a single species of organism
risk assessment	An estimate of likelihood of adverse effects that may result from exposure to certain health hazards in the environment

Risk group 1	World Health Organisation classification of microorganisms based on their relative risk. Risk group 1 microorganisms are not associated with disease in a healthy adult
risk management	The techniques of assessing, minimizing, and preventing accidental injury or illness through the use of safety measures
smear	To spread a sample of a microorganism on a glass slide in preparation for staining
sporangia	A single-celled or many-celled structure in which spores are produced, especially in fungi, algae, mosses, and ferns.
sporangiophore	a structure or stalk that bears one or more sporangia
spores	The dormant stage of certain microbial cells. They have thick walls and are able to resist and survive unfavourable environmental conditions.
sporicidal	Chemistry of a substance or product that kills spores.
sporulate	To produce or release spores
sterile	Free from disease-causing microorganisms or live bacteria
sterile paper disc	Sterile blank disc of paper used in susceptibility tests saturated with an antiseptic or disinfectant. Available from biological suppliers. May be purchased impregnated with an antibiotic.
sterilisation	The destruction of all living microorganisms including spores in or on a given environment, such as a laboratory bench, in order to prevent the spread of infection. Using heat, radiation, or chemical agents usually does this.
sterilisation indication strips 	or sterility compliance strip. Commercially available strips placed in an autoclave or pressure cooker with items to be sterilised. The strip indicates if the correct time, temperature and pressure have been reached during the run time.
streak	The propagation of microorganisms or living tissue conducive to their growth. A culture is drawn across agar in a zigzag fashion with a wire loop carrying the inoculum.
transmission	The conveyance of disease from one person to another.
viable	Capable of living, developing, or germinating under favourable conditions.
wild microbe	A microbe that is grown from an unknown source such as those found in environmental sampling. A microbe that has not been identified in the laboratory. Also referred to as environmental microbe.
yeast	Any of various one-celled fungi that reproduce by budding and can cause the fermentation of carbohydrates, producing carbon dioxide and ethanol. Some varieties of yeast are used in making beer and wine, other yeasts are pathogenic to humans.
zone of inhibition	The clear region around the paper disc saturated with antimicrobial agent on the agar surface.

Glossary references

'All about agar', Science Buddies website, http://www.sciencebuddies.org/science-fair-projects/project_ideas/MicroBio_Agar.shtml

'Cultivation Media for Bacteria', Michigan State University website, http://learn.chm.msu.edu/vibl/content/differential/

'Cytoplasmic streaming', Encyclopaedia Britannica website, http://www.britannica.com/science/cytoplasmic-streaming

Dictionary.com, Dictionary.com

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'Everyday Compounds: Sodium hypochlorite', Compound Interest, http://www.compoundchem.com/2014/07/06/sodium-hypochlorite-bleach-swimming-pools-cleaning-products/

'Identifying and using hospital grade disinfectants', CleanLink website, http://www.cleanlink.com/hs/article.aspx?id=16318

Oxfordreference.com. www.oxfordreference.com

'Physarum polycephalum', Encyclopaedia of Life website, http://eol.org/pages/1002810/overview

'Spill response accessories', Seton Australia website, http://www.seton.net.au/media/technicalinformation/MB219.pdf

The Free Dictionary. www.thefreedictionary.com

ATTACHMENT 1

Standard Operating Procedures (SOPs)

1. Examining life in pond water
2. Examining mushroom spores
3. Fermentation of yeast
4. Gram stain of a microbial culture smear
5. Growing fungi on bread
6. Making yoghurt
7. Microbes are everywhere
8. Operating a pressure cooker and autoclave
9. Pasteur's experiment
10. *Physarum polycephalum* (slime mould) care and use
11. Preparing a bacterial lawn culture
12. Preparing agar plates
13. Streak plate inoculation
14. Susceptibility testing
15. Use and care of the compound light microscope

STANDARD OPERATING PROCEDURE:

Examining life in pond water

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

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1. Introduction

Life in pond water consists of small animals that can be viewed macroscopically or microscopically. Different samples of water consist of differing animals from different habitats. This activity looks at how living things satisfy their needs for food, water and air.

2. Context

- This activity aligns with **School Level 1** of Science ASSIST Microbiology Guidelines.
- Science teachers and technicians with no specialised micro training can supervise this practical activity.
- Students must be closely supervised when undertaking this activity

3. Safety notes

- Due to the unknown microorganisms present in the water sample it is essential that staff and students wash their hands before leaving the laboratory.

4. Regulations, licences and permits

Not applicable

5. Equipment

- Cavity Slide
- Coverslip
- Dropping pipettes
- Compound Light Microscope
- Sample of pond water – available from biological suppliers or freshly collected from a pond, river, lake or aquarium.

Collecting your own sample of water

- Screw top jars
- Fine muslin net (dip net) with a long handle

Water samples may be taken from rivers, lakes, ponds, or dams. A responsible adult should collect the water sample.

Select a watercourse that is not polluted or contaminated. Sweep the net through water at different areas, depths, close to the edge and away from the edge of the water, near vegetation and clear of vegetation. Some water creatures exist in different habitats of the water course.

Place the water sample in the screw top jars. Dispose of the sample after class by either returning to the original water source if possible or by flushing down the sink.

6. Operating procedure

1. Macroscopically examine the pond life in the screw top jar. Record observations.
2. Set up the microscope. See Science ASSIST SOP: Use and care of the compound light microscope.
3. Place one drop of pond water on a cavity slide using a plastic dropping pipette.
4. Place a coverslip over the cavity of the slide.
5. Examine at 10x and then 40x magnification. Refer to pond identification sheets for pictures of common pond organisms. Look for protozoans, small animals, insects and arthropods, Examples include rotifers, vorticella, euglena, amoeba, worms, bivalves, flatworms, snails, insects and insect larvae. Include pictures of common pond water organisms. Perhaps in table form with some key features noted. Allow students to draw what they see.
6. Examine the structure and function of any microorganisms:
   - Size, shape and form of the microbes
   - Mode of locomotion (amoeboid, flagellating, cilia),
   - Organelles visible
   - Discuss the habitat—water temperature, amount of light available, if the microbe lives in the sediment or amongst water plants.

7. Trouble shooting/emergencies

- None applicable

8. Waste disposal

- Glass slides and coverslips should be disposed in a sharps container.
- Water sample disposed of into the sink.

9. Related material

- Risk Assessment.
- Further information and identification keys can be found at:

'The Aquatic Microfauna', Australian Waterlife website, http://australianwaterlife.com.au/microfauna.html (Accessed November 2016)

Math/Science Nucleus. 2004. *Guide to identification of freshwater microorganisms,* Maths/Science Nucleus website, (Accessed November 2016) http://www.msnucleus.org/watersheds/mission/plankton.pdf

'Pond identification sheet', Biology Corner website, http://www.biologycorner.com/worksheets/identifypond.html (Accessed November 2016)

References:

Pearson Heinemann. 2009. *Science Ways 1*, Pearson Australia: Port Melbourne, Victoria.

Pearson Australia. 2012. *Pearson Science 8,* Pearson Australia: Port Melbourne Victoria

Miller R. 1996. *Freshwater Invertebrates.* Gould League of Victoria, Morrabbin Victoria

STANDARD OPERATING PROCEDURE:

Examining mushroom spores

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

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1. Introduction

Fungal spores are microscopic. However, we can see the pattern they make on paper when they are released from the gills of the mushroom by making a spore print. Fungi play a vital role in many ecosystems as decomposers. Fungi such as mushrooms release digestive enzymes into their environment through their hyphae, breaking down dead matter and absorbing the nutrients.

2. Context

- This activity aligns with **School Level 1** of the Science ASSIST Microbiology Guidelines
- This practical activity can be supervised and organised by science teachers and technicians with no specialised micro training.
- Students must be closely supervised when undertaking this activity

3. Safety notes

- Students with asthma or allergies to yeasts or moulds, mushrooms or spores should wear PPE (apron, safety glasses, gloves, face mask) when participating in this activity
- Only use mushrooms purchased from the supermarket. Many wild mushrooms can be poisonous.
- Wash benches with warm soapy water after this activity.
- Always wash hands thoroughly after handling mushrooms or the spores.
- Mushrooms should not be consumed in the laboratory.

4. Regulations, licences and permits

Not applicable

5. Equipment

- Large mature mushrooms e.g. Portobello purchased from the supermarket.
- A4 black and/or white cardboard/paper
- Plastic bowl or cup
- Zip-lock bags sized to fit the card/paper
- *Optional:* microscope, slide and coverslip, knife, hairspray.

6. Operating Procedure

1. Look at the underside of the mushroom. There are thin lines radiating out from the stem to the edge. These are called gills, and are what distinguish mushrooms from other fungi. If they're a dark colour, such as brown or black, use white card. If they're a light colour, such as orange or yellow, use black card.
2. Place your sheet of card or paper flat on a bench in a location where it won't be disturbed.
3. Carefully pluck the stem from the mushroom, or use the knife to trim it. If the edge of the mushroom curls underneath, trim it back just enough to expose the mushroom's underside.
4. Place the mushroom onto the card/paper, bottom-side down. Cover the mushroom cap with a plastic bowl. This stops air currents from spoiling the print.
5. Leave for a minimum of 2 hours however best results are obtained by leaving them overnight.
6. Remove the plastic bowl.
7. Carefully lift the mushroom from the card and look at the pattern it left behind.

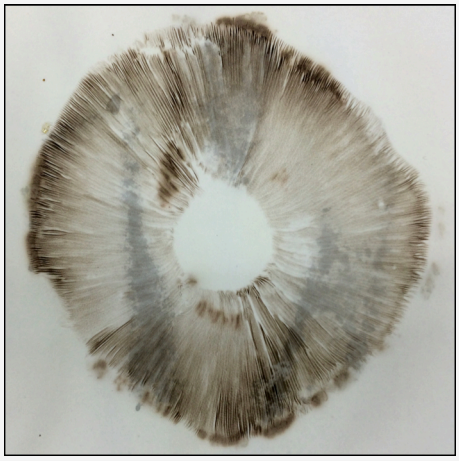

Figure 1: Mushroom spore print from a brown mushroom

8. Spore prints can be preserved by spraying them gently from about 30cm away with hairspray. Several coats are usually required. Be careful that you do not disturb the shape of the print with the pressure of the spray. Allow to dry.
9. Carefully place the spore print into a zip-lock bag and seal
10. Clean the workbench with hot soapy water.

Mushrooms can release microscopic spores into the air which float away and settle to form new mycelia elsewhere. The flower patterns on the card are these spores showing the mushroom's gill pattern. Mycologists (scientists who study fungi) can identify the type of mushroom from the colouring of the spores from these prints.

11. *To study the spores with a microscope*: Scrape off some of the spores from the spore print with a knife, and place the spores on a microscope slide.
12. Place a drop of water on the spores and cover with a cover slip. Spores are best viewed at 400x magnification. For further information on microscope use: SOP: Use and care of the compound light microscope

Operating procedure cont…

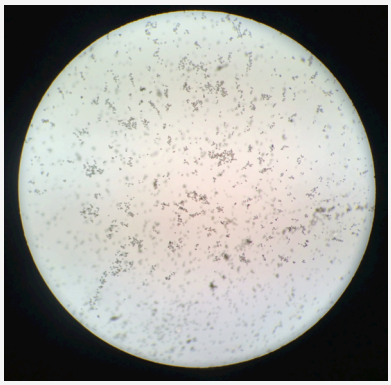

Figure 2: Shows microscopic spores from a Portobello mushroom magnified to 400x

7. Trouble shooting/emergencies

- First Aid:
  - Ingestion or inhalation of spores may cause a hay-fever type reaction. Move the student to fresh air, have assessed by first aid officer or school nurse.

8. Waste disposal

- Mushrooms should be disposed of in general refuse
- Dispose of microscope slide and coverslip in sharps container

9. Related material

- Risk Assessment.

References:

'Mushroom flowers', CSIRO Education website, http://www.csiro.au/en/Education/DIY-science/Biology/Mushroom-flowers (February 2015)

'Activity 4: Mushroom detectives', British Mycological Society website, http://www.britmycolsoc.org.uk/education/resources-and-materials/primary-school-indoor/introduction-fungi/activity-4-mushroom-detectives/ (Accessed November 2016)

'How to make a spore print', North American Mycological Association website. http://www.namyco.org/spore_prints.php (Accessed November 2016)

STANDARD OPERATING PROCEDURE:

Fermentation of yeast

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

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1. Introduction

Like all living things, yeast requires specific conditions in which to ferment and become active. When used in food production particularly bread making, the yeast must be at a certain temperature to grow and make the bread rise. This activity examines the fermentation of yeast and asks what is the best temperature for yeast to grow.

2. Context

- This activity aligns with **School Level 1** of Science ASSIST Microbiology Guidelines.
- Science teachers and technicians with no specialised micro training can supervise this practical activity.
- Students must be closely supervised when undertaking this activity.

3. Safety notes

- Students with allergies to yeast or yeast products should not participate in this activity.
- The teacher or responsible adult should handle boiling water.

4. Regulations, licences and permits

Not applicable

5. Equipment

Per group of students:

- Dry yeast available from retail stores
- 3 round balloons
- 3 small plastic drink bottles with lids
- Water – cold, warm and boiling
- Thermometer
- Funnel
- ½ teaspoon measure
- 1 teaspoon measure
- ½ cup measure
- Sugar
- Marking pen
- Tape measure

6. Operating procedure

1. Collect all equipment and label the bottles; cold, warm, boiling.
2. For each bottle, using the funnel, place ½ teaspoon of yeast and 1 teaspoon of sugar.
3. Using the funnel, add ½ cup of cold water to the bottle labelled 'cold'. Stretch a balloon over the mouth of the bottle and mix the contents gently. Place the bottle in a safe place on the bench and observe.
4. Using the funnel, add ½ cup of warm water to the bottle labelled 'warm'. Stretch a balloon over the mouth of the bottle and mix the contents gently. Place the bottle in a safe place on the bench and observe.
5. Using the funnel, the teacher or responsible adult adds ½ cup of boiling water to the bottle labelled 'boiling'. Stretch a balloon over the mouth of the bottle and mix the contents gently. Place the bottle in a safe place on the bench and observe.
6. Observe the three bottles after 30 minutes, 1 hour and 1½ hours. Measure the balloon size with a tape measure each time. Record the balloon sizes for each time.
7. Discuss the effect of temperature on balloon size over time.

7. Trouble shooting/emergencies

- First Aid:
    - In case of hot water burn, cool with cool running water for at least 20 minutes before assessment by first aid officer or school nurse

8. Waste disposal

- Dry waste including balloons, should be placed in the general refuse bin
- Liquid waste should be flushed down the sink with copious water.

9. Related material

- Risk Assessment.

References:

Primary Connections. 2007. *Marvellous Micro-organisms,* Australian Academy of Science Canberra: ACT

Australian Red Cross. *iPhone app*

Jacaranda Science. 1999. *Jacaranda Science 2.* John Wiley & Sons Australia Ltd. Milton Qld

STANDARD OPERATING PROCEDURE:

Gram stain of a microbial culture smear

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment. Before proceeding, ensure that this activity is permitted within your school jurisdiction.

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1. Introduction

The Gram stain is one of the most important and widely used differential stains in the science of microbiology. It divides bacteria into two broad groups: Gram positive and Gram negative species The basis of Gram's staining method is the ability or otherwise of a cell stained with crystal violet to retain the colour when treated with a differentiating agent, usually alcohol. Bacteria that retain the violet/purple colour are called Gram-positive. Those that lose the colour, i.e. Gram-negative, are stained in the contrasting colour of a counterstain, usually pink/red. The reaction of bacteria to the Gram stain is due to differences in the cell wall thickness and structure of the cells. It is also one of the first characteristics used to identify an unknown bacterial culture.

A 'smear' of bacteria is made on a microscope slide, fixed, stained, dried and, without using a coverslip, examined with the aid of a microscope. Aseptic technique must be observed when taking samples of a culture for making a smear. A smear that is thin and even enables the shape and arrangement of cells to be clearly seen and ensures that the staining procedure is applied uniformly.

2. Context

- This activity aligns with **School Level 3** of Science ASSIST Microbiology Guidelines.
- Science teachers and technicians who are highly trained in microbiological techniques should supervise this practical activity.
- Additional staff are recommended to enable a high level of student supervision when undertaking this activity.

3. Safety notes

- Do not commence work unless a site-specific risk assessment is completed and control measures are implemented.
- Always practice aseptic technique when working with microorganisms.
- Aerosols must be controlled to minimise their production especially on open benches. When using a loop, draw it from the cooler to the hottest parts of the Bunsen burner flame.
- Use only risk group 1 microorganisms and treat all cultures as potentially pathogenic.
- All bench surfaces should be disinfected with 70% v/v ethanol prior to and after handling microorganisms.
- Use caution when working with a Bunsen burner, the lip of the culture tubes may be hot.

- Wash hands thoroughly with soap and water before commencing activity and leaving the laboratory.
- Wear PPE: safety glasses and a disposable apron.
- Gloves are worn if there is a cut or wound on the hand, and discarded after use with infectious laboratory waste. The cut or wound should also be covered with a band aid or other dressing.
- Crystal violet, Ammonium oxalate and Iodine are toxic substances. Care should be taken to avoid ingestion, inhalation or dermal contact.
- Crystal violet is a suspected carcinogen.
- Ethanol is highly flammable.
- Follow all laboratory safety guidelines.

4. Regulations, licences and permits

Consult the science guidelines in your jurisdiction for policies regarding the subculture of microorganisms.

5. Equipment

- PPE (disposable apron or lab coat, safety glasses, gloves)
- 70% v/v ethanol
- Digital balance
- Distilled water
- Glass storage bottles for stains
- Gram stain reagents: may be purchased ready to use from scientific suppliers or prepared in the lab.
- Crystal violet stain - Crystal violet solid, Ammonium oxalate solid, ethanol
- Lugol's Iodine – Iodine solid, Potassium iodide solid
- 95% ethanol
- Safranin O stain – safranin solid, ethanol
- Bunsen burner
- Risk group 1 microorganism in nutrient broth culture or an agar plate, prepared aseptically or purchased commercially. Suggest Escherichia coli K-12 strain or Micrococcus luteus. Alternatively, a thin smear of yoghurt can be used.
- Inoculating loop
- Microscope slide
- Microscope
- Immersion oil

6. Operating Procedure

Preparation of Gram stain reagents by technician or teacher:

1. Label GHS compliant glass dropper bottles:

   Crystal Violet, Lugols Iodine, 95% ethanol, Safranin O.

2. Crystal Violet preparation:

   Solution A: 2.0g crystal violet
   20mL 95% ethanol
   Dissolve crystal violet in ethanol

   Solution B: 1.0g Ammonium oxalate
   100mL distilled water
   Dissolve ammonium oxalate in distilled water

   Mix Solution A and Solution B. Store in a labelled glass bottle

3. Lugols Iodine preparation:

   - 1.0g Iodine
   - 2.0g Potassium iodide
   - 300mL water

   Add potassium iodide and iodine to water. Allow to stand for 24 hours to allow the iodine to dissolve. Store in refrigerator in a dark glass bottle – iodine solution deteriorates with light and age.

4. 95% ethanol preparation:

   Mix 95mL of ethanol with 5mL distilled water.

5. Safranin O stain preparation:

   - 2.5g safranin O
   - 100mL 95% ethanol
   - 75mL distilled water

   Dissolve safranin in ethanol. Mix 25mL of safranin/ethanol solution with 75mL water. Filter the solution through filter paper. Store in a labelled glass bottle.

Preparation of smear by students (Alternatively, teachers or lab technicians may prepare a smear and fix slide for students)

1. Prepare lab by shutting all windows and doors to prevent draughts and the entrance of staff and students.
2. Remove all non-essential books and equipment and disinfect the bench with 70% alcohol and paper towel. Allow alcohol to evaporate and ensure all alcohol vapours have dissipated before lighting Bunsen burners
3. Collect all equipment required including cultures, slides and inoculating loop.
4. Light Bunsen burner to create a sterilising flame and provide an updraft of the surrounding area to help reduce contamination

Operating procedure cont…

5. Place the culture sample (broth or agar plate) or yoghurt, the inoculating loop, clean microscope slide near the base of the Bunsen burner. If using a broth culture check that the lid of the bottle or tube is loose.
6. Flame the inoculating loop by exposing the entire wire loop down to the end in the hottest part of the flame until it glows red for a few seconds. This will sterilise the loop. Do not put the loop down on the bench. Cool the loop by holding it near the Bunsen for 10 seconds. This prevents air contaminants from landing on the equipment.
7. If using a culture from an agar plate:
    a. Using a flamed inoculating loop, place one loopful of tap water on the centre of the slide. Reflame the loop.
    b. Find a single colony on the plate and sample only a small portion of it using the loop.
    c. Place the culture onto the drop of water on the slide and mix gently, making a smear the size of a 5 cent piece. See Figure 1.

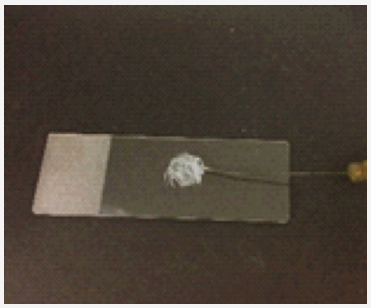

Figure 1: Spread the culture on to a slide.

8. If using a broth culture or yoghurt:
    a. Open the culture tube holding the lid in your little finger – do not put it on the bench.
    b. Flame the opening of the tube by passing it once through the Bunsen flame. Collect one loopful of culture and reflame the opening of the culture tube. Replace the lid.
    c. Place on the centre of the slide and make a smear about the size of a 5cent piece. Reflame the loop. See Figure 1

    It is important that the smear is not too thin (too few organisms on the slide), or too thick (staining will not occur properly and individual cells will not be seen).
9. Allow the smear on the slide to air dry. This may take 10 minutes.

    When dry, heat-fix the smear onto the slide by passing the slide directly through the blue Bunsen flame once only. Heat-fixing ensures the smear stays on the slide and minimizes any post-mortem changes prior to staining. Do not hold the slide in the flame as this will ‘cook’ the microbes. The smear is now ready to Gram Stain.

Gram stain procedure

1. Slides are best stained over a container to collect excess reagents for ease of disposal. A food container with applicator sticks across the top of the container works well.
2. Place slides on the applicator sticks. See Figure 2 below.
3. Flood slides with Crystal violet stain for 1 minute. All cells will take up this stain. See Figure 2 below.
4. Pour off the stain and rinse slides with gently running tap water for 5 seconds.

Operating procedure cont…

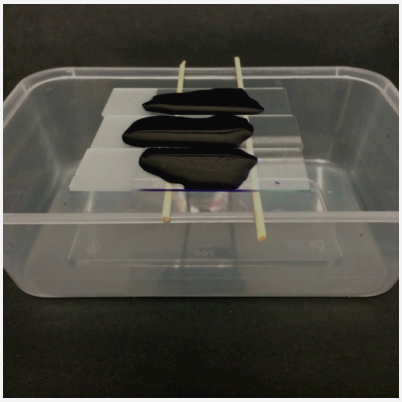

Figure 2: Flood slides with Crystal violet

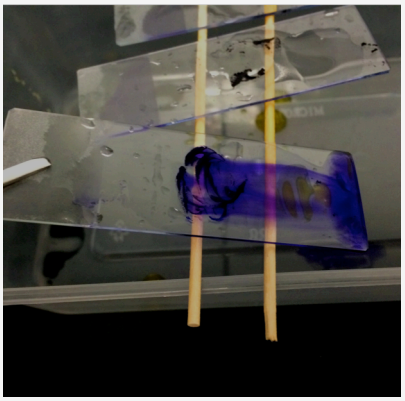

Figure 3: Decolourise with 95% ethanol

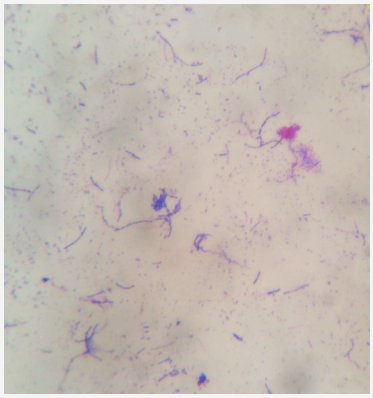

Figure 4: Gram stain of yoghurt. 1000x magnification

5. Flood slide with Lugols iodine solution for 1 minute. The iodine is a mordant and forms a dye-iodine complex to fix the dye inside the cell.
6. Poor off the stain and rinse slides with gently running tap water for 5 seconds
7. Decolourise with 95% alcohol by holding the slide with forceps at 45^0 angle and gently running the alcohol over the smear until the purple stain stops running for ~15 seconds only. See Figure 3. The decolouriser will remove the dye complex from Gram negative organisms only. This is a critical step.
8. Poor off the decolouriser and rinse slides with gently running tap water for 5 seconds
9. Counterstain with Safranin for 30–60 seconds. This stain will make the Gram negative bacteria visible.
10. Wash stain off the slide under gently running tap water
11. Allow slides to dry before examining under the microscope. Microorganisms are best observed with an oil immersion objective at 1000x magnification.

Examination of Gram stained slides

- Prepare microscope for use. See Science ASSIST SOP: Use and care of the compound light microscope.
  Gram positive: those that retain the primary basic dye and stain purple.
  Gram negative: those that loose the primary dye and stain red with the counterstain.
- Only one type of microorganism should be seen from a pure culture.
- Table 1 below shows expected Gram stain and morphology for various RG1 microorganisms.
- Figure 4 shows a Gram stain result of a yoghurt smear, which is produced with two types of microorganisms. You should see gram positive chains of cocci or diplococci (*Streptococcus thermophilus)* and rod-shaped bacilli (either *Lactobacillus acidophilus* or *L. bulgaricus)*. Any other pink globs represent milk protein such as casein.
- Students comment on the gram stain result, shape, arrangement and size of the bacterial cells.
- Gram stain results are used to plan further tests to identify a culture.

Table 1 Expected Gram stain results				
microorganism	**gram stain**	**shape**	**description**	**diagram (enlarged representation)**
Bacillus subtilis	positive	Bacillus Rod shaped	4-10µm long and ~0.25-1µm diameter Singles or in chains	
Escherichia coli	negative	Bacillus Rod shaped	~ 2µm long and ~0.25-1µm diameter Single cells	
Micrococcus luteus	positive	Coccus Spherical	irregular clusters, tetrads or pairs 1.0-1.8µm diameter	 Cocci in pairs  Cocci in tetrads  Cocci in clusters
Staphylococcus epidermidis	positive	Coccus Spherical	0.5-1.5µm diameter cocci usually form in clusters	 Cocci in clusters

7. Trouble shooting/emergencies

- First Aid: See latest SDS for more detailed information
  - In case of ingestion of ammonium oxalate, seek urgent medical attention.
  - Skin contact or eye contact from Gram stain solids or liquids should be treated by flushing with water
  - Burns should be held under cool running water for at least 20 minutes, then assessed by a first aid officer or school nurse.
  - For further advice contact the Poisons Information Centre on 131126.
- Care must be exercised to ensure that artefacts such as dirt on the slide, air bubbles in the immersion oil, undissolved salts contained with the stain and lint from lens tissue are not confused with the microorganism being observed.
- Difficult to find cells on the slide: not enough organisms in the culture, smear is too thin, organisms not properly fixed onto the slide so may have been washed away.
- Cannot see individual cells: smear is too thick.
- Mixture of Gram results: smear is too thick so the staining process has not been even for all cells on the slide e.g. not all cells have been properly decolourised, the culture is mixed or contaminated resulting in both Gram positive and Gram negative cells.
- Cell morphology distorted: excessively heat fixing the cells
- Decolourising too much or too little.

See Science ASSIST SOP: Use and care of the compound light microscope for troubleshooting problems with the microscope setup.

8. Waste disposal

- Paper towel, disposable aprons and gloves (if used) should be disposed of with contaminated waste
- Gram stain reagents are hazardous to aquatic life. Do not flush down the sink when performing staining procedure. Collect excess stain for collection by a chemical waste disposal company.
- Culture media containing microbes such as broth or agar plates should be autoclaved in their original containers prior to disposing with contaminated waste. It is very important that bottles with lids should be loosened before putting through an autoclave or pressure cooker.
- Do not autoclave paper towel of any item that has been in contact with ethanol. These can be placed into the general waste bin.

9. Related material

- Safety Data Sheets
- Risk Assessment.
- See the following Science ASSIST material on:
  SOP: Use and care of the compound light microscope.

References:

Society for General Microbiology. 2006. *Basic Practical Microbiology – A Manual.* Microbiology Online website, http://www.microbiologyonline.org.uk/file/ca2189fba3b39d24c5a44c1285d0082c.pdf

Dungey B. 2006. *The Laboratory. A science reference and preparation manual for schools.* Contemporary Press Pty Ltd, Bayswater Victoria

Chemwatch Gold. *Chemwatch Safety Data Sheet: ammonium oxalate*. http://www.chemwatch.net Subscription required (Accessed November 2015)

Chemwatch Gold. *Chemwatch Safety Data Sheet: crystal violet.* http://www.chemwatch.net Subscription required (Accessed November 2015)

Chemwatch Gold. *Chemwatch Safety Data Sheet: ethanol undenatured 70–100%.* http://www.chemwatch.net Subscription required (Accessed November 2015)

Chemwatch Gold. *Chemwatch Safety Data Sheet: iodine.* http://www.chemwatch.net Subscription required (Accessed November 2015)

Chemwatch Gold. *Chemwatch Safety Data Sheet: potassium iodide* http://www.chemwatch.net Subscription required (Accessed November 2015)

Chemwatch Gold. *Chemwatch Safety Data Sheet: safranin O.* http://www.chemwatch.net Subscription required (Accessed November 2015)

STANDARD OPERATING PROCEDURE:

Growing fungi on bread

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

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1. Introduction

Fungal spores are around us all the time, floating in the air. When they land on food, they can eventually grow into a fungus, causing the food to decay or go mouldy. In a moist environment, fungi will generally grow on bread within a week.

2. Context

- This activity aligns with **School Level 1** of the Science ASSIST Microbiology Guidelines.
- This practical activity can be supervised by science teachers and technicians with no specialised micro training
- Students must be closely supervised when undertaking this activity

3. Safety notes

- Always wash hands thoroughly after handling food items
- Bread or other food items should not be consumed in the laboratory.
- Foods are placed in zip lock bags to prevent the transmission of spores. The zip lock bags should remain zipped (closed) throughout the activity and never reopened. This avoids the inhalation of fungal spores.

4. Regulations, licences and permits

Not applicable.

5. Equipment

- PPE (disposable apron or lab coat, safety glasses, gloves)
- 3 pieces of bread - Use the same brand of bread throughout the activity
- 3 good quality resealable plastic bags – zip-lock bags are ideal
- Permanent marker
- Water
- *Optional:* other foods such as fruits, vegetables or cheese may be substituted for or tested alongside the bread samples for mould growth – cut these into 2cm cubes. Do not use foods such as meat or fish.

6. Operating Procedure

1. Label each zip-lock bag with ‘dark’, ‘fridge’, or ‘bench’ with a marker.
2. Sprinkle a small amount of water on each slice of bread.
3. Place one slice of bread in each of the three bags. Make sure each bag is sealed.
4. Take one bag and put it in a dark place – in a dark cupboard.
5. Place the next bag in the refrigerator.
6. Place the last bag on a bench in an area with lots of sunlight.
7. Leave the bags in their location for several days to up to 2 weeks, depending on the temperature and conditions in your local area.
8. Observe each bag daily to record any changes. Students can keep notes, describe and draw or photograph the fungi that grow on the different slices of bread.
9. The bread mould fungus, *Rhizopus stolonifera* is the most common fungi in the world. It has a grey/black colour. Another common mould is Penicillium a grey/green fungus often with white edges.
10. Teachers, technicians and students should thoroughly wash their hands with soap and water after the activity and before leaving the laboratory.

7. Trouble shooting/emergencies

- First Aid:
  - Ingestion or inhalation of fungal spores may cause asthma, allergy or hay-fever type reaction. Move the student to fresh air, have assessed by first aid officer or school nurse.
- If a spill occurs during the experiment (bags leaking or tearing), students must report this to their teacher immediately. Care should be taken if any aerosols or spores are released during a spill. If this occurs it may be advisable to exit any students from the laboratory that may be at risk due to asthma or allergy. If the spill is large disinfecting the surface may be necessary. Benches should be wiped with a suitable disinfectant eg.1% solution of sodium hypochlorite, or 70% alcohol. Both have good activity on mould. Wear disposable gloves and mask to decontaminate the area.
- For further advice contact the Poisons Information Centre on 131126.

8. Waste disposal

- Bread slices should remain in the closed zip lock bags when the activity is complete, and the bags not reopened.
- Place the zip lock bags in a garbage bag and dispose in general refuse.

9. Related material

- Risk Assessment.

- See Science ASSIST questions on: Students investigating mould and bacterial growth on food items

References:

'Bread, Mold, Fungus'. Backyard Nature website, http://www.backyardnature.net/f/bredmold.htm (Accessed November 2016)

'Activity: Bread Mold Experiment'. Education.com website, http://www.education.com/activity/article/Making_Mold_Science_Experiment/ (Accessed November 2016)

'Student activity: Growing fungi on bread', Science Learning website, http://sciencelearn.org.nz/Contexts/Hidden-Taonga/Teaching-and-Learning-Approaches/Growing-fungi-on-bread (Accessed November 2016)

'Mold Bread Experiment', Explorable website, https://explorable.com/mold-bread-experiment (Accessed November 2016)

'Mold Terrarium', Exploratorium website, https://www.exploratorium.edu/science_explorer/mold.html (Accessed November 2016)

STANDARD OPERATING PROCEDURE:

Making yoghurt

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

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1. Introduction

Yoghurt is a fun way to look at the use of microbes in food production. Yoghurt is basically milk fermented with a specific strain of bacteria (usually *Lactobacillus acidophilus* and *Streptococcus thermophilis*) that turns the lactose into lactic acid and so the milk into yoghurt. The lactic acid is what causes the milk to thicken and taste tart.

You can use any kind of milk, including whole milk, 2 per cent, 1 per cent, non-fat, pasteurised, homogenised, organic, raw, diluted evaporated, dry powdered, cow, goat, soybean, and more.

2. Context

- This activity aligns with **School Level 1** in the Science ASSIST Microbiology Guidelines.
- Science teachers and technicians with no specialised micro training can supervise this practical activity.
- Students must be closely supervised when undertaking this activity.

3. Safety notes

- Food should not be consumed if this activity is carried out in a science lab.
- If it desired to eat the yoghurt, then an alternative room should be arranged such as the home economics area which has facilities that follow safe food handling procedures. In this way, activities can be conducted where the food may be eaten.
- Students with allergies to cow's milk or milk products should not participate in this activity.

4. Regulations, licences and permits

Not applicable.

5. Equipment

Per group of students:

- 100mL UHT milk
- 1 tablespoon skim milk powder
- 1/8 teaspoon of yoghurt starter purchased from a yoghurt supply company or some store-bought, plain yogurt with active yogurt cultures.
- 250mL glass jar with an airtight lid suitable for fermenting and storing yoghurt
- Thermometer – food grade
- Saucepan
- Tablespoon

- Teaspoon
- Measuring cup (1 cup)
- Fork
- Plastic kitchen wrap
- Hot plate
- Access to a refrigerator

6. Operating procedure

1. The technician or teacher should sterilise jars before class.
    - Sterilise clean jars in the oven. Place jar and lid upside down in an oven set at 100°C for 10 minutes.
    - Alternatively, place the jars and lids in a lidded pot and boil for 10 minutes. Let cool before removing.
2. Bring the yoghurt starter to room temperature before the start of the class.
3. Place 100mL UHT milk and a thermometer in a saucepan. Heat to 43°C while stirring with a spoon on a stovetop or hot plate.
4. Remove the pan from the heat, add about 1 tablespoon of skim milk powder to the warm milk, and stir with a fork. Make sure it fully dissolves.
5. Add yoghurt starter to the milk. You will need about 1/8 teaspoon of yoghurt starter or 2 tablespoons of plain yoghurt with active culture per cup (250mL) of milk. Stir in with a fork
6. Pour the mixture into the sterile jar and cover with plastic wrap or screw the lid on.
7. Allow the yoghurt to incubate overnight. Keep the yogurt warm and still to encourage bacterial growth, while keeping the temperature as close to 38°C as possible. Use an esky with warm water, or sit the jar in a warm area such as on a hot water heater or hot plate. Keep the yoghurt still during incubation. Jiggling won't ruin it, but it makes it take a lot longer to set.
8. Pour the whey (thin yellow liquid) off the top of the yoghurt then test to check that it has set. Gently press the bowl of a teaspoon into the surface of the yoghurt. If it leaves an indent, it has set.
9. Refrigerate the yogurt for several hours before serving. It will keep for 1 to 2 weeks. If you are going to use some of it as a starter culture, use it within 5 to 7 days so that the bacteria still have growing power.
10. Your yoghurt is ready to enjoy. If this activity has been conducted in a home economics kitchen the yoghurt can be eaten as is, or add strawberry topping, honey, applesauce or fruit. **It is not to be eaten if it has been prepared in a science laboratory.**

7. Trouble shooting/emergencies

- First Aid:
    - In case of heat burns, hold affected area under cool running water for at least 10 minutes. Have the burn assessed by school nurse or GP.

- In case of cuts from broken glass, carefully remove any shards of glass, and cover wound with a pressure bandage to stop bleeding.
- In case of allergies, check with students for known sensitivity to dairy products prior to commencing activity. An alternate activity may be best for these students. If swelling around the lips, face or hands, runny eyes or vomiting or diarrhoea occurs, refer immediately to school nurse or call 000.

- Yoghurt not setting properly:
  - Unsterile jars may have been used. These can allow the growth of other bacteria which will affect the thickness and taste of the yoghurt.
  - Old starter culture.

8. Waste disposal

- Discard any leftover ingredients with general refuse.

9. Related material

- Risk Assessment.
- See a previously answered question by Science ASSIST: Eating in Labs

References:

'How to make homemade yoghurt'. How to make your own yoghurt website, http://www.makeyourownyogurt.com/make-yogurt/what-you-need (Accessed November 2016)

'How to make yoghurt', Local Harvest website, http://www.localharvest.org.au/learn/how-to-make-yoghurt/ (Accessed November 2016)

St John Ambulance Australia. 1998. *Australian First Aid.* Impact Printing: Melbourne

'Yoghurt Cultures', Science Buddieswebsite, http://www.sciencebuddies.org/science-fair-projects/project_ideas/MicroBio_p010.shtml (February 2015)

STANDARD OPERATING PROCEDURE:

Microbes are everywhere

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

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1. Introduction

Microbes exist all around us – on the human body, on animals and in the environment. This activity enables students to explore locations and growth patterns of microbes around us. Good hygiene practices should be observed at all times. Teachers and laboratory technicians should consult the microbiological directives in their jurisdiction prior to undertaking microbiological practical activities.

2. Context

- This activity aligns with **School Level 2** of the Science ASSIST Microbiology Guidelines.
- Science teachers and technicians who are trained in sterilisation and decontamination procedures should supervise this practical activity.
- Students must be closely supervised when undertaking this activity.

3. Safety notes

- Good hygiene practices should be observed at all times.
- Keep hands away from mouth, nose, eyes and face during and after this activity.
- Wash hands immediately before and after handling agar plates and cotton swabs.
- Do not take swabs from areas likely to contain pathogens such as toilet areas and human body fluids or skin.
- Teachers and laboratory technicians should consult SOP: Preparing agar plates for safety advice when preparing agar plates for this activity.
- Regard all microorganisms as potential pathogens, and treat them with standard microbiological practices to minimise risk to laboratory staff and the environment.
- Care must be taken if there is a great deal of condensate in the lid of the agar plate. This can easily drip out carrying microbes with it.
- Decontaminate workbenches with disinfectant (70% ethanol or hospital grade disinfectant) before and after microbiological activities.
- Count plates out and in.
- Sodium hypochlorite (bleach solution) is a strong oxidiser. Wear PPE and neoprene gloves when preparing.
- Plates should be sealed around the whole circumference before student viewing to reduce the risk of students opening the plates

4. Regulations, licences and permits

Not applicable.

5. Equipment

- PPE: safety glasses, lab coat, closed shoes
- 6 nutrient agar plates per group of students
- 3 sterile cotton swabs or cotton buds per student group
- Sodium hypochlorite bleach solution 0.5–1% v/v (5000–10000 ppm) (available chlorine) in a beaker per student group
- Marking pen or wax pencil
- Sticky tape or laboratory sealing film (<1cm wide)
- Disinfectant for laboratory benches. 70% ethanol or hospital grade general purpose disinfectant (the label on the front of the pack must state 'hospital grade', which is a general purpose hard surface disinfectant which will kill microorganisms)
- 3 x 2–3mL sterile water in a test tube or Bijou bottle per group of students.
- Paper towel

6. Operating procedure

1. Determine which area each student is to sample e.g. window sill, benchtop etc.
2. Disinfect the laboratory bench with paper towel and either 70% alcohol or hospital grade general disinfectant if is the area is not to be sampled.
3. Collect all equipment:
    - 1 sterile nutrient agar plate per student for each sample area, an air exposure plate and a control plate.
    - 1 sterile swab for each sample area
    - 1 test tube containing sterile water. The use of sterile water enables the cotton tip to run smoothly over the agar without gouging it.
    - 1 beaker containing 0.5–1% v/v (5000–10000 ppm) (available chlorine) sodium hypochlorite (bleach)
    - Sticky tape or laboratory sealing film
4. Open one of the plates in an area of the lab for 20 minutes. This is an air exposure plate. Another plate should not be opened but sealed ready for incubation. This represents a control plate.
5. Label the base of another agar plate around the edge not in the middle where any growth could be obscured with student name, date and area to be sampled. Place the culture plate upside down on the bench so that the lid is on the bench and the base of the plate containing the agar is uppermost.
6. Gently tear the swab packaging at the handle end so that the handle is slightly exposed. See figure 1. Alternatively, carefully take one clean cotton bud from pack.

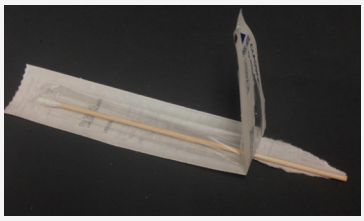

Figure 1. Sterile cotton swab stick with handle exposed immediately prior to sampling

Operating procedures cont…

7. Remove the sterile cotton swab from the packaging without touching the cotton tip and immediately moisten the cotton tip in the sterile water. Run the moistened cotton tip over the surface of the sample area for approximately 2–5 seconds.
8. Immediately pick up the base of the culture plate with your left hand and turn it so that the agar surface is facing you. Swab the agar surface with the cotton tip in a rolling motion or in a backwards and forwards motion over the entire area of the agar. This must be done very gently so the swab doesn't break the agar. See Figure 2.

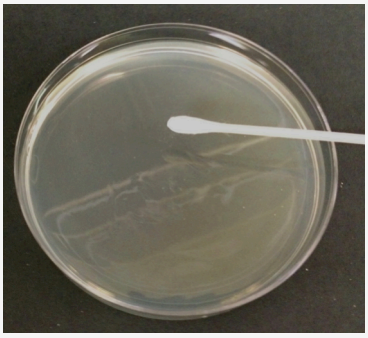

Figure 2: Gently roll the swab across the surface of the agar

9. Immediately replace the culture plate into the lid of the Petri dish.
10. Tape the agar plate closed using four pieces of sticky tape at 12 o'clock, 3 o'clock, 6 o'clock and 9 o'clock. Alternatively, use one strip of laboratory sealing film by wrapping it once around the perimeter of the agar plate where the two halves join. The laboratory sealing film must not overlap at any section.
11. Incubate the plates for 24 to 48 hours at room temperature. The plates should be placed in a secure area, flat on a bench with the agar side upper most, away from sunlight. Alternatively the plates can be placed into an incubator set at 25^{o}C.
12. After incubation, the plates should **not be reopened** and should be sealed around the whole circumference to reduce the risk of students opening the plates before distribution to students for examination.
13. Students should be able to visually differentiate the mixed colonies growing on the surface of the agar. A description of several colonies can be performed using the guide provided in Chapter 9. Each colony represents thousands or millions of microorganisms. Immediately after the activity, students should return the plates to the teacher for sterilisation and disposal in an autoclave or pressure cooker (15psi, 121^{o}C for 15–20 minutes).
14. Disinfect laboratory benches with 70% alcohol.
15. Wash hands and dry thoroughly.

7. Trouble shooting/emergencies

- First aid: See latest SDS for more detailed information
  - **If swallowed:** Do not induce vomiting. Rinse mouth with water, and then give water to drink. Seek urgent medical attention.
  - **If in eyes:** Hold open and irrigate with copious quantity of water for at least 15 minutes. Seek medical attention.

- **If on skin/clothes:** If spilt on skin or clothes quickly wipe off with a dry cloth to absorb as much liquid as possible. Remove contaminated clothes and drench the area with excess water under a safety shower. Seek medical attention.
- **If inhaled:** Remove to fresh air and seek medical attention if symptoms persist.
- For further advice contact the Poisons Information Centre on 131 126.

8. Waste disposal

- Agar plates containing microbial growth should be sterilized in an autoclave or pressure cooker (15psi, 121^{o}C for 15–20 min) prior to disposal in the general waste bin.
- 0.5-1% Sodium hypochlorite should be disposed of by flushing to sewer with copious water. Check with your local jurisdiction.

9. Related material

- Risk assessment.
- Safety Data Sheets for Sodium hypochlorite bleach, disinfectant and nutrient agar.

References:

'Microbes all around us', Nuffield Foundation website, http://www.nuffieldfoundation.org/practical-biology/microbes-all-around-us (Accessed November 2016)

Chemwatch Gold. 2013. *Long Safety Data Sheet: Bleach solution*. http://www.chemwatch.net Subscription required. (Accessed November 2016)).

ASSIST AUSTRALIAN SCHOOL SCIENCE INFORMATION SUPPORT FOR TEACHERS AND TECHNICIANS

STANDARD OPERATING PROCEDURE:

Operating a pressure cooker and autoclave

Note: To be undertaken only by trained personnel in conjunction with a site-specific risk assessment and the manufacturer's instructions.

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1. Introduction

A pressure cooker, pressure steam steriliser or an autoclave is commonly used in the laboratory to effectively sterilise micro-organisms and agar. Pressure steam sterilisers and autoclaves reach the recommended temperature and pressure required to render most micro-organisms and agar sterile.

2. Context

These instructions are for the use of experienced science teachers and technicians only. These guidelines are general and the manufacturer's user manual should always be consulted for the particular equipment in use.

3. Safety notes

- Very hot surfaces – use a sign where appropriate to indicate hot surfaces.
- Steam – burns and scalds hazard; always open pressure cooker lid away from face.
- Very hot liquids.
- Heavy equipment.
- High pressure; only open pressure cooker when cooled and the pressure is down to zero.
- Must be stored and operated in the science preparation room or dedicated microbiology preparation area only away from students.
- Do not leave pressure cookers unattended during use.

4. Regulations, licences and permits

Pressure cooker

To maintain sterilization at 121^{0}C for 15–20 minutes at 15p.s.i (pounds per square inch), the following considerations must be given when purchasing a pressure cooker.

- Ensure the pressure cooker is an adequate size to sterilise agar in bottles.
- The pressure cooker must contain a pressure gauge and be able to reach a pressure of 15p.s.i. (103kPa) and a temperature of 121^{0}C.

Note that some domestic cookers do not meet these requirements.

Autoclave

An autoclave manufacturer or supplier will advise you on the appropriate autoclave type for your purpose. Ensure the autoclave manufacturer has preset programmes for your specific sterilising needs and provides metal racks and/or baskets for sterilising small items.

5. Equipment

- Pressure cooker or autoclave
- Oven bags for pressure cooker or autoclave bags
- Chemical sterility indicator strips in the form of paper or cardboard strips. The use of autoclave tape is an indication that an article has gone through a heating process but should not be relied upon as a control of sterilisation
- PPE, including heat proof gloves, safety glasses or face shield
- Distilled water
- Heat source or power supply for pressure cooker
- Power supply for autoclave
- Glassware that is heat resistant borosilicate. Bottles should have appropriate heat resistant lids and glass conical flasks plugged with non-absorbent cotton wool

6. Operating procedure

General notes for pressure cooker and autoclave use

Preparation of articles to be sterilised.

- Check that materials and equipment are able to withstand high temperatures and pressure. Inspect glassware for chips or cracks.
- Conical flasks or bottles of agar should be filled to no more than 2/3, plugged with non-absorbent cotton wool plugs covered loosely with aluminium foil or greaseproof paper to prevent the plug from becoming wet. Lids on bottles should be loosened by ¼ turn.
- Forceps and other instruments should be wrapped loosely in aluminium foil or greaseproof paper (optionally wrapped in foil) to permit the penetration of steam into the pack.
- Use only autoclavable test tube racks.
- To allow for steam penetration, autoclave bags must not be tightly packed or sealed, nor should they be reopened.
- Place chemical sterility indicator strips in the centre of every load. Check the strip after the run is complete to ensure temperature and steam conditions have been met.
- Autoclaving is not recommended for radioactive wastes because of the potential for contaminating equipment and generating radioactive vapours. You should also never autoclave solvents, volatile or corrosive chemicals. Oils are usually sterilised by dry heat.
- Do not use a pressure cooker in an oven.
- Only use distilled water (if available) in the autoclave or pressure cooker. Tap water contains minerals that build-up in the unit and will need to be removed regularly.
- Do not stack or store combustible material next to an autoclave (cardboard, plastic, volatile or flammable liquids).
- Do not attempt to open the lid or door while pressure cooker or autoclave is operating.
- After sterilisation, make sure the pressure is down to zero before opening either the pressure cooker or autoclave. Avoid touching the inner chamber surfaces.
- Qualified persons should only perform repair and servicing of pressure cookers or autoclaves.

Operating procedure continued…..

- For spilled material clean up.

1. Review the Safety Data Sheet (if appropriate) to determine the protective equipment, spill cleanup and disposal protocols that are necessary.
2. Wait until the pressure cooker or autoclave and articles have cooled to room temperature.
3. Contain any spilled material using paper towel to absorb or contain the spill.
4. Clean the equipment and work area in order to collect and remove all spilled materials.
5. Dispose of the waste following the protocol appropriate for the material. If materials have been intermingled, follow the cleanup and disposal protocol for the most hazardous component of the mixture

Pressure cooker operation

1. Inspect the vent pipe and holes in the vent nut of the lid, the black rubber over pressure plug and the sealing ring before using each time.
2. Place the cooker on a level heatproof surface, over a heat source or close to a power point.
3. Add approximately 2cm of distilled water to the base of the cooker to cover the support dish. Ensure that the support dish and internal heating element (if applicable) are covered.
4. Place prepared articles to be sterilised on the support stand in the cooker—do not exceed manufacturers recommended load capacity, generally 2/3 full. Ensure there is space around items for the steam to circulate.
5. Grease the point of contact between the lid and the base with silicone grease before each use to ensure a good seal.
6. Place the lid on the cooker and ensure it is locked into position and tightly sealed. Commence heating the cooker by warming on the medium to high heat setting. Check the control valve is in the open position initially, to allow the release of steam to exhaust the air that is trapped in the cooker. When the air has been exhausted, close the control valve. If your pressure cooker has a pressure regulator weight, allow the steam to vent prior to putting on the regulator weight. Depending on the brand of cooker, the plunger will rise or a needle on the pressure gauge will move to indicate the rise in pressure. The lid will now remain locked while the cooker is under pressure.
7. The correct pressure has been reached when the pressure regulator begins to rock, or the pressure gauge reaches the green sterilisation zone. Sterilisation time can now commence. Maintain the temperature at 121^0C for 15–20 minutes.
8. When sterilisation time is complete, turn the pressure cooer off and allow the pressure to reduce. The lid can be removed when the pressure has dropped to zero. Do not cool the cooker under cold water. Wear heatproof gloves and safety glasses or face shield to slowly remove the lid locking devices.
9. Remove and check the chemical indicator strip according to manufacturer's instructions. When sterilisation has been established, remove sterilised material. Waste material should be double bagged and placed in the general waste bin.
10. Remove all water from the steriliser prior to storing. Do not leave water in the steriliser overnight. Store in a dry area.

Operating procedure continued…..

Autoclave operation

1. Check autoclave water volume. Use distilled water to fill. Check that the door seal is not compromised, the drain seal at the bottom of the chamber is clear of debris, and ensure the waste bottle is empty.
2. Insert metal trays into the chamber.
3. Add prepared articles to be autoclaved, ensuring the load does not exceed manufacturer's recommendations and steam is able to freely circulate around the articles.
4. Turn the autoclave power on and close the door, ensuring the door is sealed.
5. Set the temperature to 121^{0}C, pressure at 15p.s.i. (pounds per square inch) for 15–20 minutes. Start the cycle.
6. When the cycle is complete, an alarm will sound. If fitted, turn the autoclave to vent. The door should not be opened until the pressure has reduced to zero. If a drying stage is provided, a drying process is activated and operates until the load is dry.
7. Allow sterilised material to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.
8. Stand back from the door as a precaution, carefully open door to no more than 2.5 cm to release residual steam and allow pressure within liquids and containers to normalise. Do not agitate containers of super-heated liquids or remove caps before unloading.
9. Remove and check the chemical indicator strip according to manufacturer's instructions. When sterilisation has been established, remove autoclaved articles with heatproof gloves and wear safety glasses or a face shield.
10. After removal from the autoclave, place hot items in an area which clearly indicates the items are 'hot' until the items cool to room temperature.
11. Record in a log book the date, duration and operator for each time the autoclave is run.
12. Trained service personnel should carry out monitoring and validation according to the manufacturer's instructions at 12-month intervals.

7. Trouble shooting/emergencies

- First aid:
  - Burns and scalds: If clothing is soaked in hot water/steam, remove clothing and any jewellery and cool the injured body area under running cool water for at least 20 minutes. Cover the area with a clean, non-adhesive dressing and seek medical attention for a burns assessment.
  - In case of biological exposure, wash skin with soap and water.

8. Waste disposal

- It should be noted that pressure cookers and autoclaves are used for sterilising and do not produce waste as such, but all microbial waste materials should be sterilised and double bagged before disposing in the regular waste.
- Sterilised inoculated agar plates should be disposed of in the unopened sterilising bag. Double bag and place into general waste.

- Unused sterilised agar should be allowed to set, and then double bagged and then placed in general waste. Do not allow agar to set in drains.

9. Related material

- Manufacturers instruction manual
- Risk assessment
- SDS for materials to be sterilised and the products produced.

References:

Centre for Healthcare Related Infection Surveillance and Prevention 2008. *Disinfection and Sterilization Control Guidelines. Section 4 Quality Management in Sterilization* Queensland Department of Health website http://www.health.qld.gov.au/chrisp/sterilising/section_4.pdf

'E8.015 – Steroclave, 15L. non-electric, 31cm base diametre' Southern Biological websitehttp://www.southernbiological.com/kits-and-equipment/microbiology-equipment-and-supplies/sterilisers/e8-015-steroclave-15l-non-electric-31cm-base-diameter/ (Accessed June 2015)

'Pressure Cooker Instruction Manuals' Pressure Cooker Centre website, http://www.pressurecooker.com.au/Pressure-Cooker-Instruction-Manuals (Accessed June 2015)

Siltex Australia Pty Ltd 2009. *Pratika B/Pratika S quick reference guide.* Siltex (Australia) East Bentleigh, Victoria.

St John Ambulance 2010. *Australian First Aid Manual* St John Ambulance: Barton, ACT.

STANDARD OPERATING PROCEDURE:

Pasteur's experiment

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

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1. Introduction

Pasteur's experiment demonstrates that microbes are present in air and on dust and require a suitable environment in which to grow. The use of a 'S' tube shows how contamination could be prevented.

2. Context

- This activity aligns with **School Level 2** of the Science ASSIST Microbiology Guidelines.
- Science teachers and technicians who are trained in sterilisation and decontamination procedures should supervise this practical activity.
- Students must be closely supervised when undertaking this activity.

3. Safety notes

- Good hygiene practices should be observed at all times.
- Keep hands away from mouth, nose, eyes and face during and after this activity.
- Wash hands immediately before and after handling broth.
- Care should be exercised when handling glass test tubes, tubing, hot water and broth.

4. Regulations, licences and permits

Not applicable.

5. Equipment

- PPE: safety glasses, lab coat, closed shoes
- Nutrient broth. Alternatively low salt chicken or beef stock
- 2 x 25mL test tubes in a test tube rack
- 2 x cotton wool plugs – tightly roll a wad of non-absorbent cotton wool to securely fit the mouth of the test tube
- 1 x 10–15cm straight glass tube 8mm diameter
- 1 x 'S' shaped glass tube 8mm diameter
- Marker pen

6. Operating procedure

1. Prepare nutrient broth following the manufacturer's instructions.
2. While the broth is still boiling, allocate approximately 15mL to each tube labelled # 1 and # 2.
3. Plug the mouth of each test tube with the cotton wool plug.
4. Insert the straight tube through the cotton wool plug in the tube labelled #1. Ensure it is secure, with one end exposed out of the top of the cotton wool plug, and the other end in the tube or flask but not in the broth.
5. Insert the 'S' tube through the cotton wool plug in the tube labelled #2. Ensure it is secure, with one end exposed out the top of the cotton wool plug, and the other end in the tube or flask but not in the broth.
6. Observe the colour and opacity of the broth in each tube at 0 minutes.
7. Observe the colour and opacity of the broth in each tube after 24 hours and at 48 hours. If bacteria have entered the tube, the broth will become cloudy after 24 hours.
8. The broth should be sterilised in an autoclave and then washed to waste.
9. All equipment should be sterilised and thoroughly washed after use.

7. Trouble shooting/emergencies

- First aid: See latest SDS for more detailed information
  - **If swallowed:** Do not induce vomiting. Rinse mouth with water, and then give water to drink. Seek urgent medical attention.
  - **If in eyes:** Hold open and irrigate with copious quantity of water for at least 15 minutes. Seek medical attention.
  - **If on skin/clothes:** If spilt on skin or clothes quickly wipe off with a dry cloth to absorb as much liquid as possible. Remove contaminated clothes and drench the area with excess water under a safety shower. Seek medical attention.
  - **If inhaled:** Remove to fresh air and seek medical attention if symptoms persist.
  - For further advice contact the Poisons Information Centre on 131 126.
- Cut skin from broken glass should be washed well, any glass removed if it is practical to do so, and a pressure bandage applied to stem bleeding.

8. Waste disposal

- Broth solutions may be disposed of in the sink with copious water.

9. Related material

- Safety Data Sheet for nutrient broth, if used.
- Risk Assessment.

References:

Wymer P. 1987. *Practical Microbiology and Biotechnology for Schools,* MacDonald: London

Chemwatch Gold. Chemwatch *Safety Data Sheet: nutrient* broth. http://www.chemwatch.net Subscription required. (Accessed February 2016)

'Louis Pasteur Experiment: Refute Spontaneous Generation', Pasteur Brewing website, http://www.pasteurbrewing.com/louis-pasteur-experiment-refute-spontaneous-generation/ (Accessed November 2016)

'Recreation of Pasteur's Experiment', Messengale's Biology Junction website, http://biologyjunction.com/pasteur_experiment.htm (Accessed November 2016)

STANDARD OPERATING PROCEDURE:

Physarum polycephalum (slime mould) care and use

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

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1. Introduction

Physarum polycephalum is a slime mould that grows in dark humid conditions under the bark of decaying trees and amongst leaf litter on the forest floor. It is used as a tool for demonstrating cytoplasmic streaming* locomotion to students. *Physarum polycephalum* is purchased as a living organism and needs to be fed daily and subcultured to prevent it from outgrowing the petri dish. The plasmodium is the active feeding stage of the organism and consists of a mass of multinucleate protoplasm. In moving, the plasmodium may move along many fronts that are connected by veins. Streaming of protoplasm is easily seen within the veins.

2. Context

- This activity aligns with **School Level 3** of the Science ASSIST Microbiology Guidelines.
- This practical activity should be supervised by science teachers and technicians who are highly trained in microbiological techniques.
- Additional staff are recommended to enable a high level of student supervision when undertaking this activity.

3. Safety notes

- *Physarum polycephalum* is a slime mould that is noted as a microorganism that is suitable for use in schools[1]. It is not known to be toxic.
- The Petri dish should remain closed during class demonstrations.
- *Physarum polycephalum* is a living culture that, if allowed to starve or dry out, may begin to sporulate*. The spores* are unlikely to generate microbial aerosols*.
- Wear gloves, safety glasses and lab coat/apron when handling.
- Regard all microorganisms as potential pathogens, and treat them with standard microbiological practices to minimise risk to laboratory staff and the environment.

4. Regulations, licences and permits

Not applicable

5. Equipment

For handling and cultivating

- PPE: safety glasses, gloves, lab coat, closed shoes
- A clean, non-traffic area to feed and subculture
- 70% ethanol (flammable)
- Rolled oats
- Sterile scalpel blade

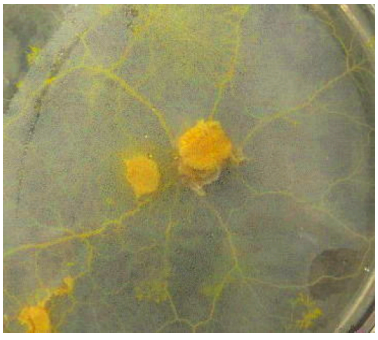

Figure 1 *Physarum plasmodium*

- Sterile forceps
- Fresh sterile plain agar plate
- Lab sealing tape
- Physarum should be stored at room temp away from bright light.

For class demonstrations

- PPE: safety glasses, gloves, lab coat, closed shoes
- Light microscope or stereo microscope
- Optional: Digital display camera for whole class viewing.
- Viable sample of *Physarum polycephalum* in a lidded Petri dish.

6. Operating procedure

Feeding the *Physarum* culture – daily

1. Disinfect the bench and equipment with 70% ethanol (flammable)
2. Using clean forceps, place 1-2 rolled oats onto the plain agar on the bright yellow area/s.
3. Immediately replace the lid.
4. Store in a dark area at room temperature. Petri dish may be wrapped in foil if no dark area is available.
5. Disinfect the bench and equipment with 70% ethanol (flammable)

Subculturing *Physarum polycephalum*

Physarum polycephalum may be subcultured approximately every 5-7 days to prevent the culture from outgrowing the Petri dish and to increase the number of Petri dishes available for classroom use.

1. Disinfect the bench and equipment with 70% ethanol (flammable)
2. Using a sterile scalpel, select an area that has a colonised oat flake. Cut an agar cube (approx $1cm^3$) encompassing the colonised area and carefully lift the section out of the Petri dish with sterile forceps. Replace the lid.
3. Immediately place the agar cube onto a fresh plain agar plate with the colonised oat flake in direct contact with the agar. Close the lid and tape closed with lab sealing tape.
4. Repeat steps 2 and 3 if desired, using a fresh sterile plain agar plate each time.
5. Feed the sub-cultured sample after 24 hours as per instructions above.
6. Close and seal the Petri dish with lab sealing tape.
7. Disinfect the bench and equipment with 70% ethanol (flammable)

Cytoplasmic streaming class demonstration

1. Setup a light microscope or stereo microscope.
2. Place a Petri dish containing the *Physarum polycephalum* on the microscope stage and remove the lid.
3. Close the diaphragm or lower the sub-stage condenser of the microscope.
4. Focus on the yellow branches of the Physarum using the 4x objective (40x magnification). Observe the back and forth flow of the cytoplasm within the yellow branches. The flow will change direction every few minutes.

7. Trouble shooting/emergencies

- First aid: See latest SDS for more detailed information
  - **If swallowed:** Do not induce vomiting. Rinse mouth with water, and then give water to drink. Seek medical attention.
  - **If in eyes:** Hold open and irrigate with copious quantity of water for at least 15 minutes. Seek medical attention.
  - **If on skin/clothes:** Wash affected area with copious quantities of water immediately. Remove contaminated clothes and wash before reuse. If swelling, redness, blistering or irritation occurs seek medical attention.
  - **If inhaled:** Remove to fresh air and seek medical attention if symptoms persist.
  - For further advice contact the Poisons Information Centre on 131126
  - *Any health concerns should be referred to the school first aid officer for assessment, accompanied by the relevant latest SDS if applicable. Follow your school's accident and incident policy and reporting procedures.*

8. Waste disposal

- *Physarum polycephalum* can be maintained after class use for an extended period of time. However, the ongoing commitment required to feed and subculture the culture is time consuming.
- Cultures should be sterilized by autoclave or pressure cooker at 121^0C, 15psi for 15-20 minutes prior to disposing with general waste.

*Glossary:

- **Cytoplasmic streaming** - the movement of the fluid substance (cytoplasm) within a plant or animal cell. The motion transports nutrients, proteins, and organelles within cells.
- **Microbial Aerosols** – A fine mist of water droplets that contain microorganisms.
- **Plasmodium** - A mass of protoplasm having many cell nuclei but not divided into separate cells it is formed by the combination of many amoeba-like cells and is characteristic of the active, feeding phase of certain slime moulds.
- **Spores** – The dormant stage of certain microbial cells. They have thick walls and are able to resist and survive unfavourable environmental conditions.
- **Sporulate** – to produce or release spores

Related material:

- Risk assessment
- Safety Data Sheets for 70% ethanol
- Science ASSIST example risk assessment for *Physarum polycephalum* care and use.

Further ideas for use of *Physarum polycephalum* in the laboratory

Methods for Chemotaxis and Phototaxis:

Bozzane, Donna M (2001), 'Cells with "Personality": *Physarum polycephalum*', *Carolina Tips* August 2001 Vol. 64, No.3, Southern Biological website, http://file.southernbiological.com/Assets/Products/Specimens/Living_Specimens_and_Supplies/Plants_and_Fungi/L2_30-Physarum_slime_mould_culture/CarolinaTipsPhysarum.pdf

Experimenting with food types and conditions for growth:

'L2.30 – Physarum sline mould, live', Southern Biological website, http://www.southernbiological.com/specimens/living-specimens-and-supplies/plants-and-physarum/l2-30-physarum-slime-mould-live/ (Accessed November 2016)

References:

[1] Microbiology in schools advisory committee. 2010. *Suitable and unsuitable micro-organisms.* Microbiology in schools advisory committee. Reading, UK. http://www.misac.org.uk/PDFs/MiSAC_suitable_and_unsuitable_micro-organisms.pdf

Bozzane, Donna M (2001), 'Cells with "Personality": *Physarum polycephalum*', *Carolina Tips* August 2001 Vol. 64, No.3, Southern Biological website, http://file.southernbiological.com/Assets/Products/Specimens/Living_Specimens_and_Supplies/Plants_and_Fungi/L2_30-Physarum_slime_mould_culture/CarolinaTipsPhysarum.pdf

CCH Australia Ltd (2011) 'CCH Australian Laboratory Safety Manual', Documents 35-110 Organisation, responsibility and planning of work, Documents 35-130 Classification of microorganisms by risk group.

Latty, T. ARC Postdoctoral Fellow, Behaviour and Genetics of Social Insects Lab, School of Biological Sciences, University of Sydney, Telephone conversation April 2014.

Online Dictionary, http://www.britannica.com (Accessed April 2014)

Southern Biological. 2009. *Physarum polycephalum*. Southern Biological website. http://file.southernbiological.com/Assets/Products/Specimens/Living_Specimens_and_Supplies/Plants_and_Fungi/L2_30-Physarum_slime_mould_culture/L2_30_Physarum_CareInstructions.pdf (2009)

History of reviews

Date	Version Number	Notes
Nov 2014	Version 1.0	
Dec 2016	Version 2.0	Amended description of *Physarum polycephalum* to align with MISAC document and Science ASSIST Microbiology guidelines for Australian schools Amended glossary items to be consistent with Science ASSIST Microbiology guidelines for Australian schools Removed references to plasmodial fusion

STANDARD OPERATING PROCEDURE:

Preparing a bacterial lawn culture

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment. Before proceeding, ensure that this activity is permitted within your school jurisdiction.

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1. Introduction

A bacterial lawn culture is a set of bacterial colonies that form a confluent growth or mat of bacteria on an agar plate. It can be used to test susceptibility to antibiotics, antiseptics and disinfectants. Aseptic technique should be observed whenever micro-organisms are transferred from one container to another. Sterile equipment and media should be used to transfer and culture micro-organisms. Contaminated equipment should preferably be heat sterilised by either incineration or autoclaving.

2. Context

- This activity aligns with **School Level 3** of Science ASSIST Microbiology Guidelines.
- Science teachers and technicians who are highly trained in microbiological techniques should supervise this practical activity.
- Additional staff are recommended to enable a high level of student supervision when undertaking this activity.

3. Safety notes

- Do not commence work unless a site-specific risk assessment is completed and control measures are implemented.
- Always practice aseptic technique when working with microorganisms.
- Aerosols must be controlled to minimise their production especially on open benches. When using a loop, draw it from the cooler to the hottest parts of the Bunsen burner flame.
- Use only risk group 1 microorganisms and treat all cultures as potentially pathogenic.
- All bench surfaces should be disinfected with 70% ethanol prior to and after handling microorganisms.
- Use caution when working with a Bunsen burner, the lip of the culture tubes may be hot.
- Wash hands thoroughly with soap and water before commencing activity and leaving the laboratory.
- Wear PPE: safety glasses and a disposable apron.
- Gloves are worn if there is a cut or wound on the hand, and discarded after use with infectious laboratory waste. The cut or wound should also be covered with a band aid or other dressing.
- Care must be taken if there is a great deal of condensate in the lid of the agar plate. This can easily drip out carrying microbes with it.
- Follow all laboratory safety guidelines.

4. Regulations, licences and permits

Consult the science guidelines in your jurisdiction for policies regarding the subculture of microorganisms.

5. Equipment

- PPE (disposable apron or lab coat, safety glasses)
- 70% alcohol
- 0.5-1% bleach solution. 1 beaker per group of students
- Risk group 1 microorganism in nutrient broth culture, prepared aseptically or purchased commercially. Suggest Escherichia coli K-12 strain or Micrococcus luteus.
- 1 sterile nutrient agar plate
- 1 sterile plastic dropping pipette
- 1 sterile hockey stick spreader
- Bunsen burner
- Sticky tape or laboratory sealing film
- Incubator or suitable area for incubation that is isolated, away from sunlight and 24–30^{0}C.

6. Operating procedure

1. Prepare lab by shutting all windows and doors to prevent draughts and the entrance of staff and students.
2. Remove all non-essential books and equipment and disinfect the bench with 70% alcohol and paper towel. Allow alcohol to evaporate and ensure all alcohol vapours have dissipated before lighting Bunsen burners
3. Collect all equipment required including cultures, agar plates, dropping pipette and hockey stick spreader.
4. Light Bunsen to create a sterilising flame and provide an updraft of the surrounding area to help reduce contamination
5. Working close to the Bunsen burner, take up the broth culture bottle in the left hand and hold the lid with the little finger on the other hand. Rotate the bottle to remove the lid and keep hold of the lid so that it does not touch the bench. Flame the opening of the bottle by passing it through the flame several times. This creates an expansion of air in the tube to minimise microorganisms entering the tube. Do not put the cap down.
6. Aseptically remove the dropping pipette from its wrapping, and take up 2–3 drops of culture. Replace the lid of the tube.
7. The agar plate should be placed agar side down on the bench. Remove and hold the lid slightly open at an angle, opening away from your body. Keep the petri dish open for the minimum amount of time to reduce the risk of contamination.
8. Place 1–2 drops of culture medium onto the agar. Immediately place the dropping pipette into the bleach container. Put the lid back onto the Petri dish.

Operating procedure cont…

9. Aseptically remove the sterile wrapping of the hockey stick spreader, remove and hold the lid of the Petri dish and gently spread the culture over the whole of the agar surface to distribute the bacteria evenly across it, then remove the hockey stick spreader. Close the petri dish and put the hockey stick spreader into the container of bleach solution to kill the remaining bacteria. Use this cultured plate for susceptibility testing of antiseptics and disinfectants. See SOP: Susceptibility testing of antiseptics and disinfectants. Alternatively:
10. Replace lid and seal with one strip of laboratory sealing film wrapped once around the circumference of the agar plate, or 2-4 short pieces of sticky tape to hold the lid on to the base.
11. Incubate the Petri dish with the agar side uppermost at 24-30^{0}C for 24-48 hours in an aerobic environment. This can be in an incubator or an area in the lab away from direct sunlight.
12. Decontaminate the bench with 70% alcohol.
13. 13. Remove PPE and place into an autoclave or oven bag. Wash hands before leaving the laboratory or starting another activity.
14. 14. After incubation, plates can be stored in the refrigerator again with the agar side uppermost for up to 5 days.
15. These plates should remain sealed whilst students examine them for an even and complete spread of growth over the surface of the agar plate (a lawn). Have the students comment on the growth.

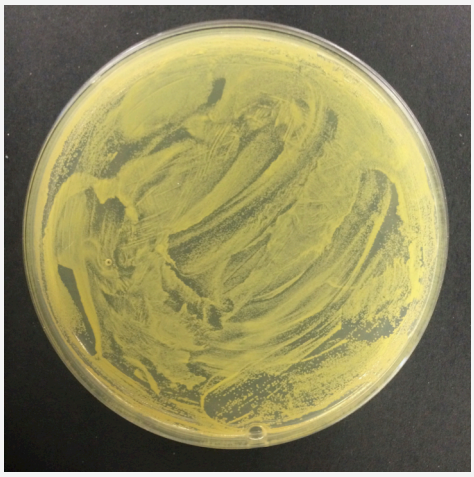

Figure 1: Lawn culture *Micrococcus lutes* on nutrient agar

16. After the plates have been examined, autoclave or sterilise at 121^{0}C, 15psi for 15–20 minutes before double bagging and disposing in general refuse.

7. Trouble shooting/emergencies

- First Aid: See latest SDS for more detailed information
  - If swallowed: Do not induce vomiting. Rinse mouth with water, and then give water to drink. Seek medical attention.

  - If in eyes: Hold open and irrigate with copious quantity of water. Seek medical attention.
  - If on skin or hair: Flush skin and hair with running water (and soap if available). Seek medical attention if irritation occurs.
  - For further advice contact the Poisons Information Centre on 131126.

- No lawn culture produced: Insufficient culture picked up with the swab and placed onto the surface of the agar.
- Different shapes, sizes and coloured colonies growing on the surface of the agar plate: there is contamination on the plate. This may have resulted from incorrectly prepared and sterilised nutrient agar and/or broth culture containing the RG1 microorganism. Poor aseptic technique allowing unwanted microorganisms to contaminate the previously sterilised material.

8. Waste disposal

- Dropping pipettes should be placed in a 0.1% bleach solution for 2 hours prior to disposing into the regular waste.
- Hockey stick spreaders should be placed in a 0.1% bleach solution for 2 hours prior to washing in warm soapy water, then resterilising when dry.
- Paper towel, disposable aprons and gloves (if used) should be placed into an autoclave or oven bag and sterilised at 15psi, 121^{o}C for 15–20 minutes, before being disposed of in the regular waste.
- Culture media containing microbes such as broth or agar plates should be autoclaved in their original containers prior to disposing in the regular waste. It is very important that bottles with lids should be loosened before putting through an autoclave or pressure cooker.
- Do not autoclave paper towel of any item that has been in contact with ethanol. These can be placed into the general waste bin.

9. Related material

- Safety data sheets for microorganisms used, nutrient agar, bleach and ethanol
- Risk Assessment.
- See the following Science ASSIST material on:
  SOP: Operating a pressure cooker and autoclave
  SOP: Preparing agar plates
  Agar plate experiments

References:

Society for General Microbiology. 2006. *Basic Practical Microbiology – A Manual.* Microbiology Online website, http://www.microbiologyonline.org.uk/file/ca2189fba3b39d24c5a44c1285d0082c.pdf

'Aseptic Techniques', Nuffield Foundation website, *http://www.nuffieldfoundation.org/practical-biology/aseptic-techniques* (Accessed November 2016)

'Making a spread or 'lawn' plates', Nuffield Foundation website, http://www.nuffieldfoundation.org/practical-biology/making-spread-or-%E2%80%98lawn%E2%80%99-plate (November 2011)

STANDARD OPERATING PROCEDURE:

Preparing agar plates

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

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1. Introduction

Nutrient agar is suitable for most microbiology practicals involving bacteria performed in schools. Plain agar is a suitable culture media for the cultivation of *Physarum polycephalum* (slime mould). The preparation of agar plates must be carried out aseptically (under sterile conditions) to ensure all plates are initially free of micro-organisms and other contaminants. Nutrient agar plates must only be used to culture organisms permitted in your jurisdiction and must not be opened after culturing. When used for culturing, agar must be set (like edible jelly) and at room temperature.

2. Context

- This activity aligns with **School Level 2** of Science ASSIST Microbiology Guidelines.
- This practical activity should be supervised by science teachers and technicians who are trained in sterilisation and decontamination procedures.
- Students must be closely supervised when undertaking this activity.

3. Safety notes

- Maintenance: All equipment must be maintained according to manufacturer's instructions
- Handling: Attach warning signage to hot equipment. Follow good laboratory practice by limiting the handling of hot equipment and thoroughly washing hands after handling.
- Storage: Ensure the lid of the jar of purchased nutrient agar is tightly sealed before storing.
- Labelling: Write the preparation date in small numerals at the edge of the plate.

4. Regulations, licences and permits

Not applicable

5. Equipment

- PPE (disposable apron, safety glasses)
- Steriliser, pressure cooker or autoclave
- Balance
- Measuring cylinder
- Conical flask with non-absorbent cotton wool plug, or Schott bottle - sized to hold the required amount
- Hotplate stirrer and magnetic flea (stirring bar)
- Petri dishes: sterile plastic Petri dishes, 90mm diameter are recommended for ease of use

- Disinfectant: 70% ethanol for cleaning benches before and after pouring agar into plates. Prepare 70% ethanol by measuring 70mL ethanol (or methylated spirits) and making up to 100mL with distilled/deionised water. Note: 70% Ethanol is flammable.
- Nutrient agar (Commercially available agar with premixed nutrients) or nutrient agar prepared in-house.
- Plain agar ingredients – agar and distilled water

6. Operating procedure

To make 1 litre, sufficient for approximately 50–60 plates.

1. Using a digital balance, prepare nutrient agar medium according to the manufacturer's instructions. Alternatively prepare in-house:

   - Beef extract or ground up stock cubes 5–10g
   - Peptone yeast extract 5–10g
   - Agar 15g
   - Distilled water 1L

   Plain agar prepared in-house:

   - Agar 15g
   - Distilled water 1L

2. Place water in a 1L beaker. Using the hotplate stirrer and magnetic stirring bar, mix ingredients with the water whilst stirring to prevent clumping. Heat whilst stirring until the agar dissolves at 95^{o}C. The agar is poured into conical flasks and plugged with non-absorbent cottonwool, or into Schott bottles filled no further than 2/3. The lid of the Schott bottle is to be closed fully, then released 1/4 turn prior to sterilising.
3. Sterilize the conical flask or Schott bottle using a pressure cooker or autoclave 15-20 minutes at 121^{o}C, 15psi, according to manufacturer's instructions for use of this equipment.
4. The pressure in the vessel should be at zero prior to opening the equipment. The sterilized agar is allowed to cool to 50^{o}C–55^{o}C, this is the optimal temperature for pouring to minimise condensation. It might be useful to sit the flask or bottle in a water bath at this temperature to maintain the optimal temperature prior to pouring plates. Agar solidifies at 40^{o}C.
5. Thoroughly wash and dry hands, wear a disposable apron.
6. Create a sterile area by thoroughly wiping the bench with 70% alcohol and paper towel. Do not completely dry the ethanol: allow it to evaporate. Air conditioners, fans and fume cupboards must be turned off, windows and doors closed and foot traffic restricted, to reduce the chance of contamination to plates while pouring.
7. Label the sterile Petri dishes and organise them base down (the smaller section of the two halves) on the bench.
8. Hold the flask containing sterile agar medium in liquid form with your right hand. Turn your left-hand palm side up and clamp the cotton plug between two fingers.

Figure 1: Pouring an agar plate

9. Use the hand holding cotton plug to lift the lid of the Petri dish. Pour approximately 15 to 20mL of the sterilised nutrient agar medium into the base of the Petri dish, until it is about half full. Hold the Petri dish lid so that it partially covers the bottom of the dish as you pour. This prevents microbes and air borne dust particles from dropping into the sterile plate and contaminating it. See Figure 1.
10. Immediately place the lid on the base at an angle so that steam is able to escape.
11. Pour the remaining plates the same way.

7. Trouble shooting/emergencies

- First Aid: See latest SDS for more detailed information
  - **If swallowed:** Do not induce vomiting. Rinse mouth with water, and then give water to drink. Seek medical attention.
  - **If in eyes:** Hold open and irrigate with copious quantity of water. Seek medical attention.
  - **If on skin or hair:** Flush skin and hair with running water (and soap if available). Seek medical attention if irritation occurs.
  - **If inhaled:** Remove to fresh air and seek medical attention if symptoms persist.
  - *Any health concerns should be referred to the school first aid officer for assessment, accompanied by the relevant latest SDS if applicable. Follow your school's accident and incident policy and reporting procedures.*
- Condensation of agar plates occurs when steam is trapped inside the plate when the lid has been fully replaced on the dish when the agar is too hot. Allow agar to cool to 50^0C – 55^0C before pouring.
- Agar not setting is a result of not using correct amount of agar or of not adequately dissolving the agar prior to sterilising. Check carefully that all solids have dissolved before sterilising.
- Contamination is a result of inadequate aseptic technique or contaminated benches or equipment. Review operator aseptic techniques prior to pouring agar plates.

8. Waste disposal

- Cleaning up: All equipment used in the preparation of agar is to be washed thoroughly in warm soapy water, rinsed and dried.

- All agar plates, including plates not cultured, must be sterilised/autoclaved prior to being placed in double plastic garbage bag and sealed for immediate disposal in the industrial bins

9. Related material

- Manufacturer's Safety Data Sheets
- Risk Assessment.
- Manufacturer's instructions for pressure cooker/ autoclave
- Science ASSIST AIS: Microwave, pressure cooker or Autoclave? Recommendations for best practice of sterilizing agar

References:

Chemwatch Gold. 2012. *Long Safety Data Sheet: Nutrient Agar*. http://www.chemwatch.net Subscription required. (Accessed March 2014).

Young, J. 2008. *The Biolab Sourcebook.* Triple Helix Resources: NZ

History of reviews

Date	Version Number	Notes
July 2014	Version 1.0	
Dec 2016	Version 2.0	Amended context to align with Science ASSIST Microbiology guidelines for Australian schools Added photo and recipe for plain agar

STANDARD OPERATING PROCEDURE:

Streak plate inoculation

1. Introduction

The inoculating loop is used for preparing a streak plate. This involves the progressive dilution of an inoculum of bacteria or yeast over the surface of solidified agar medium in a Petri dish in such a way that colonies grow well separated from each other. When bacterial cells are close together they produce colonies that will merge. If bacterial cells are separated enough they produce isolated colonies. The aim of this procedure is to obtain single isolated pure colonies. Each single colony will contain millions of cells that are identical to the parent cell.

2. Context

- This activity aligns with **School Level 3** of Science ASSIST Microbiology Guidelines.
- This practical activity should be supervised by science teachers and technicians who are highly trained in microbiological techniques.
- Additional staff are recommended to enable a high level of student supervision when undertaking this activity.

3. Safety notes

- Do not commence work unless a site specific risk assessment is completed and control measures are implemented.
- There must be no subculturing or opening of plates or broths inoculated by students.
- Always practice aseptic technique when working with microorganisms.
- Aerosols must be controlled to minimise their production especially on open benches. When using a loop, draw it from the cooler to the hottest parts of the Bunsen burner flame.
- Use only risk group 1 microorganisms and treat all cultures as potentially pathogenic.
- All bench surfaces should be disinfected with 70% ethanol prior to and after handling microorganisms.
- Use caution when working with a Bunsen burner, the lip of the culture tubes may be hot.
- Wash hands thoroughly with soap and water before commencing activity and leaving the laboratory.
- Wear PPE: safety glasses and a disposable apron.
- Gloves are worn if there is a cut or wound on the hand, and discarded after use with infectious laboratory waste. The cut or wound should also be covered with a band aid or other dressing.
- Care must be taken if there is a great deal of condensate in the lid of the agar plate. This can easily drip out carrying microbes with it.
- Follow all laboratory safety guidelines.

4. Regulations, licences and permits

Consult the science guidelines in your jurisdiction for policies regarding the subculture of microorganisms.

5. Equipment

- PPE (disposable apron, safety glasses)
- 70% v/v ethanol
- Risk group 1 microorganism in nutrient broth culture prepared aseptically or purchased commercially. Suggest *Escherichia coli K-12* strain or *Micrococcus luteus*.
- 1 sterile nutrient agar plate
- Bunsen burner
- Inoculating loop
- Sticky tape or laboratory sealing film
- Incubator or suitable area for incubation that is isolated, away from sunlight and 24-30^{0}C
- Paper towel

6. Operating procedure

1. Prepare lab by shutting all windows and doors to prevent draughts and the entrance of staff and students.
2. Remove all non-essential books and equipment and disinfect the bench with 70% alcohol and paper towel. Allow alcohol to evaporate and ensure all alcohol vapours have dissipated before lighting Bunsen burners
3. Place the culture broth, the inoculating loop and the sterile nutrient agar plate near the base of the Bunsen burner. The Petri dish should be placed on the bench so that the lid is on the bench and the base of the plate containing the agar is uppermost. Label the base of the plate containing the media, around the edge.
4. Loosen the lid of the broth but do not remove it. Light Bunsen burner to create a sterilising flame and provide an updraft of the surrounding area to help reduce contamination
5. Flame the inoculating loop by exposing the entire wire loop down to the end in the hottest part of the flame until it glows red for a few seconds. This will sterilise the loop. Do not put the loop down on the bench. Cool the loop by holding it near the Bunsen for 10 seconds. This prevents air contaminants from landing on the equipment
6. Working close to the Bunsen burner take up the broth culture bottle in the other hand and hold the lid with the little finger on the loop hand. Rotate the bottle to remove the lid and keep hold of the lid so that it does not touch the bench. Flame the opening of the bottle by passing it through the flame several times. This creates an expansion of air in the tube to minimise microorganisms entering the tube. Do not put the cap down.
7. Insert the loop into the broth to take up one loopful of the culture.
8. Carefully remove the loop, do not shake it and reflame the bottle opening. Holding the loop carefully, place the cap on the bottle and move it to the side.
9. Pick up the agar plate by its base; spread the inoculum over 1/4 of the surface of the plate by a series of sideways strokes of the loop back and forth across the agar. See figure 1 – marked area 1. Strokes should be placed gently on the agar. Do not gouge the agar.
10. Place the plate back on its lid and reflame the loop. Place the loop in the holder. If you are right handed, rotate the plate 75º clockwise.

Operating procedure cont….

11. When the loop has cooled, lift up the plate and use the loop to spread 4 streaks from the original inoculum into the second 1/4 of the agar – marked area 2. These strokes are spread in one direction only. Note that the inoculum being spread has been greatly diluted. Again, rotate the plate about 75º clockwise.

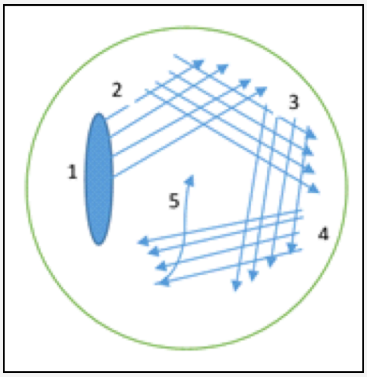

Figure1: Plan of streak pattern

12. Repeat the procedure a further 2 times, but at the end of each series of strokes remember to replace the plate on its lid, rotate the plate and reflame the loop.
13. Finally make a single streak that starts in the end of the last set of diluting streaks and zigzags across the remainder of the plate – marked area 5. This final streak should produce isolated colonies – and it is very important that this streak does not come in contact with any of the other preliminary diluting streaks or the primary inoculum
14. Place the plate back onto its lid and seal with one strip of laboratory sealing film wrapped once around the circumference of the agar plate, or 2–4 short pieces of sticky tape that hold the lid on to the base.
15. Incubate the Petri dish with the agar side uppermost at 24–30^{0}C for 24-48 hours in an aerobic environment. This can be in an incubator or an area in the lab away from direct sunlight.
16. Decontaminate the bench with 70% v/v ethanol.
17. Remove PPE and place into an autoclave or oven bag. Wash hands before leaving the laboratory or starting another activity.
18. After incubation, plates can be stored in the refrigerator again with the agar side uppermost for up to 5 days.
19. These plates should remain sealed whilst students examine them for the presence of well isolated single colonies. Have students comment on their streaking technique.
20. After the plates have been examined, autoclave or sterilise at 15psi, 121^{o}C, for 15–20 minutes before double bagging and disposing in general refuse.

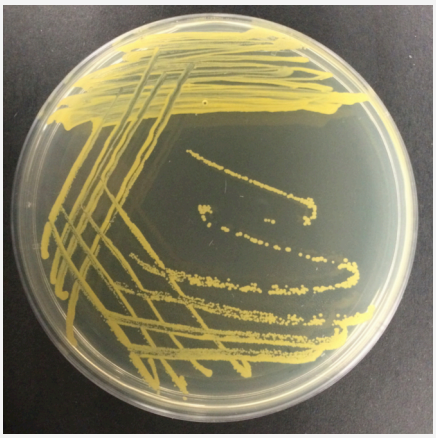

Figure 2: *Micrococcus luteus* on nutrient agar using streak plate inoculation

7. Trouble shooting/emergencies

- First Aid: See latest SDS for more detailed information
    - If swallowed: Do not induce vomiting. Rinse mouth with water, and then give water to drink. Seek medical attention.
    - If in eyes: Hold open and irrigate with copious quantity of water. Seek medical attention.
    - If on skin or hair: Flush skin and hair with running water (and soap if available). Seek medical attention if irritation occurs.
    - For further advice contact the Poisons Information Centre on 131126.
- Insufficient growth: There has been too little inoculum or a hot loop has been used which has killed the microorganism.
- Different shapes, sizes and coloured colonies growing on the surface of the agar plate: there is contamination on the plate. This may have resulted from incorrectly prepared and sterilised nutrient agar and/or broth culture containing the RG1 microorganism. Poor aseptic technique allowing unwanted microorganisms to contaminate the previously sterilised material.

8. Waste disposal

- Paper towel, disposable aprons and gloves (if used) should be disposed of with contaminated waste
- Culture media containing microbes such as broth or agar plates should be autoclaved in their original containers prior to disposing with contaminated waste. It is very important that bottles with lids should be loosened before putting through an autoclave or pressure cooker.
- Do not autoclave paper towel of any item that has been in contact with ethanol. These can be placed into the general waste bin.

9. Related material

- Safety data sheets for microorganisms used and nutrient agar
- Risk Assessment.
- See the following Science ASSIST material on:
  SOP: Operating a pressure cooker and autoclave
  SOP: Preparing agar plates
  Agar plate experiments

References:

Society for General Microbiology. 2006. *Basic Practical Microbiology – A Manual.* Microbiology Online website,
http://www.microbiologyonline.org.uk/file/ca2189fba3b39d24c5a44c1285d0082c.pdf

STANDARD OPERATING PROCEDURE:

Susceptibility testing of antiseptics and disinfectants

Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.

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1. Introduction

Antiseptics are used in the disinfection of external surfaces of living tissue. Disinfectants are used to decontaminate inanimate objects such as equipment and bench surfaces. This activity uses the Kirby-Bauer disc diffusion method of antimicrobial sensitivity testing. In this test an antiseptic or disinfectant is incorporated into a paper disc, which is placed on an agar plate containing a pure lawn culture of the organism to be tested. The antiseptic or disinfectant diffuses into the agar and can inhibit the growth of the organism if it is sensitive to the test agent, producing a clear zone of inhibition. Absence of a zone of inhibition is indicative of resistance. The zones of inhibition are measured after incubation and these values can be compared to a table with standardised data. Doctors use Kirby-Bauer test results to choose antibiotics effective against the specific bacteria causing a patient's infection.

2. Context

- This activity aligns with **School Level 3** of the Science ASSIST Microbiology Guidelines.
- Science teachers and technicians who are highly trained in microbiological techniques should supervise this practical activity.
- Additional staff are recommended to enable a high level of student supervision when undertaking this activity.

3. Safety notes

- Do not commence work unless a site-specific risk assessment is completed and control measures are implemented.
- There must be no subculturing or opening of plates or broths inoculated by students.
- Always practice aseptic technique when working with microorganisms.
- Aerosols must be controlled to minimise their production especially on open benches. When using a loop, draw it from the cooler to the hottest parts of the Bunsen burner flame.
- Use only risk group 1 microorganisms and treat all cultures as potentially pathogenic.
- All bench surfaces should be disinfected with 70% ethanol prior to and after handling microorganisms.
- Use caution when working with a Bunsen burner, the lip of the culture tubes may be hot.
- Wash hands thoroughly with soap and water before commencing activity and leaving the laboratory.
- Wear PPE: safety glasses and a disposable apron.
- Gloves are worn if there is a cut or wound on the hand, and discarded after use with infectious laboratory waste. The cut or wound should also be covered with a band aid or other dressing.

- Care must be taken if there is a great deal of condensate in the lid of the agar plate. This can easily drip out carrying microbes with it.
- Follow all laboratory safety guidelines.

4. Regulations, licences and permits

Consult the science guidelines in your jurisdiction for policies regarding the subculture of microorganisms.

5. Equipment

- PPE (disposable apron or lab coat, safety glasses)
- 70% alcohol
- 0.5-1% bleach solution. 1 beaker per group of students
- Beaker to collect contaminated forceps to be sterilised in an autoclave or pressure cooker. Do not place metal forceps in bleach as they will rust.
- Inoculate the nutrient agar plates. See SOP: Preparing a bacterial lawn culture.
- 4 pairs of sterile forceps
- Sterile paper discs (available from biological suppliers)
- Suggested antiseptics and disinfectants: liquid handwash, various concentrations of alcohol, iodine solution, hospital grade disinfectant Use no more than 4–6 discs per plate.
- Sticky tape or laboratory sealing film
- Incubator or suitable area for incubation that is isolated, away from sunlight and 24–30^{o}C

6. Operating Procedure

1. Prepare lab by shutting all windows and doors to prevent draughts and the entrance of staff and students.
2. Remove all non-essential books and equipment and disinfect the bench with 70%alcohol and paper towel. Allow alcohol to evaporate and ensure all alcohol vapours have dissipated before lighting Bunsen burners
3. Collect all equipment required including cultures, agar plates, dropping pipette and hockey stick spreader.
4. Using a permanent marker, divide the base of the agar plate into 4 sections. Label the edge of each section as Control, and 3 selected antiseptics/disinfectants
5. Light Bunsen to create a sterilising flame and provide an updraft of the surrounding area to help reduce contamination.
6. Refer to SOP: Preparing a bacterial lawn culture to produce a lawn culture on an agar plate.
7. Using the sterile forceps, dip a blank sterile disc in a solution of the first chemical agent saturating the whole disc. Drain the saturated disc on absorbent paper then place it on the inoculated agar plate in the appropriate labelled section.
8. Repeat step 7 each time with a new sterile pair of forceps for the other antiseptic/disinfectant agents. With sterile forceps, place a control disc directly onto the labelled section of the agar plate. Place forceps in a discard container to be decontaminated in the autoclave at the end of the activity.

Operating procedure cont…

9. Replace lid and seal with one strip of laboratory sealing film wrapped once around the circumference of the agar plate, or 2–4 short pieces of sticky tape to hold the lid on to the base.
10. Incubate the Petri dish with the agar side uppermost at 24–30^{o}C for 24–48 hours in an aerobic environment. This can be in an incubator or an area in the lab away from direct sunlight. The moisture on the disc will hold it in place, they will not dislodge.
11. Decontaminate the bench with 70% alcohol.
12. Remove PPE and place into an autoclave or oven bag. Wash hands before leaving the laboratory or starting another activity

After incubation, plates can be stored in the refrigerator again with the agar side uppermost for up to 5 days

Plate examination (NB The plate must remain sealed during investigation).

1. Examine the plates for a lawn growth and any signs of contamination. Look for zones of clearing around the discs. Figure 1 shows *Micrococcus luteus* with pre-impregnated discs grown on a nutrient agar plate for 24 hours at 28^{o}C.

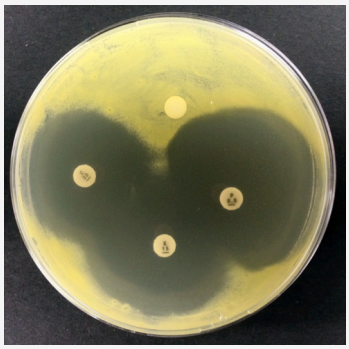

Figure 1. Sample susceptibility test result. *Micrococcus luteus* grown on nutrient agar for 24 hours at 28^{0}C.

2. Measuring the zone of inhibition around the disc from the agar side of the closed plate. See figure 2. With a millimetre ruler, measure the diameter across the zone that surrounds the disc where no bacterial growth has occurred. Record the results in a table. The bigger the zone of inhibition, the more sensitive the bacteria is to the antimicrobial agent.
3. Results may show
   - no clearing with microbial growth up to the disc is recorded as resistant to the antimicrobial agent, or
   - with a zone of clearing around the disc recorded as sensitive to the antimicrobial agent.

   Degree of positive growth may be recorded as +, ++ or +++. This is a qualitative estimate only.
4. After plates have been examined, autoclave or sterilise at 121^{o}C, 15psi for 15-20 minutes before double bagging and disposing in general refuse.
5. Decontaminate the bench with 70% alcohol.

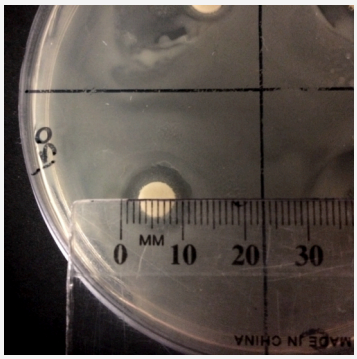

Figure 2. Measuring the zone of inhibition of iso-Propyl alcohol on *Escherichia coli* grown on a nutrient agar plate

7. Trouble shooting/emergencies

- First Aid: See latest SDS for more detailed information
    - If swallowed: Do not induce vomiting. Rinse mouth with water, and then give water to drink. Seek medical attention.
    - If in eyes: Hold open and irrigate with copious quantity of water. Seek medical attention.
    - If on skin or hair: Flush skin and hair with running water (and soap if available). Seek medical attention if irritation occurs.
    - For further advice contact the Poisons Information Centre on 131126.
- No lawn culture produced: Insufficient culture picked up with the swab and placed onto the surface of the agar.
- Different shapes, sizes and coloured colonies growing on the surface of the agar plate: there is contamination on the plate. This may have resulted from incorrectly prepared and sterilised nutrient agar and/or broth culture containing the RG1 microorganism. Poor aseptic technique allowing unwanted microorganisms to contaminate the previously sterilised material. A pure culture has not been produced.

8. Waste disposal

- Contaminated forceps should be placed into a container such as a beaker for sterilising in an autoclave or pressure cooker.
- Paper towel, disposable aprons and gloves (if used) should be placed into an autoclave or oven bag and sterilised at 15psi, 121^{o}C for 15–20 minutes, before being disposed of in the regular waste.
- Culture media containing microbes such as broth or agar plates should be autoclaved in their original containers prior to disposing in the regular waste. It is very important that bottles with lids should be loosened before putting through an autoclave or pressure cooker. Do not autoclave paper towel of any item that has been in contact with ethanol. These can be placed into the general waste bin.

9. Related material

- Safety data sheets for microorganisms, antiseptics and disinfectants used, and nutrient agar
- Risk Assessment.
- See the following Science ASSIST material on:
  SOP: Operating a pressure cooker and autoclave
  SOP: Preparing agar plates
  Agar plate experiments

References:

World Health Organisation. 1991. *Basic Laboratory Procedures in Clinical Bacteriology.* Health Library for Disasters website, http://helid.digicollection.org/en/d/Jwho01e/4.10.html (Accessed November 2016)

Society for General Microbiology. 2006. *Basic Practical Microbiology – A Manual.* Microbiology Online website, http://www.microbiologyonline.org.uk/file/ca2189fba3b39d24c5a44c1285d0082c.pdf

STANDARD OPERATING PROCEDURE:

Use and care of the compound light microscope

Note: To be undertaken only by trained personnel in conjunction with a current site-specific risk assessment.

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1. Introduction

The microscope is a tool that enables us to view things that are too small to be seen with the naked eye. The most common type of microscope used in school science laboratories is the compound light microscope. It uses a system of two or more lenses to collect and focus transmitted visible light through a specimen to the eye. It is the principle tool for the study of biology and is often referred to as *bright field microscopy*. Animal cells, plant cells, protozoa and bacteria can be easily seen with a compound light microscope. The typical compound light microscope is able to magnify from 40x to 1000x, increasing our ability to see detail so that objects as small as 0.2 micrometres (µm) or 200 nanometres (nm) can be seen. Compound light microscopes may be monocular (for viewing using only one eye) or binocular (for viewing using both eyes). Compound light microscopes from various manufacturers may appear different but operate on similar principles. A microscope is a delicate precision instrument and care must always be used when using, transporting and maintaining it.

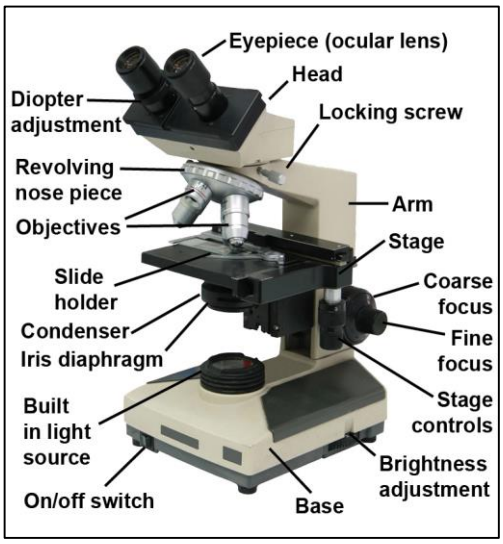
A typical school microscope has three magnifications: Scanning, Low and High. Each objective and eyepiece (ocular lens) will have the magnification written on it. Some microscopes will also have an oil immersion objective.

The total magnification is the ocular magnification multiplied by the objective magnification.

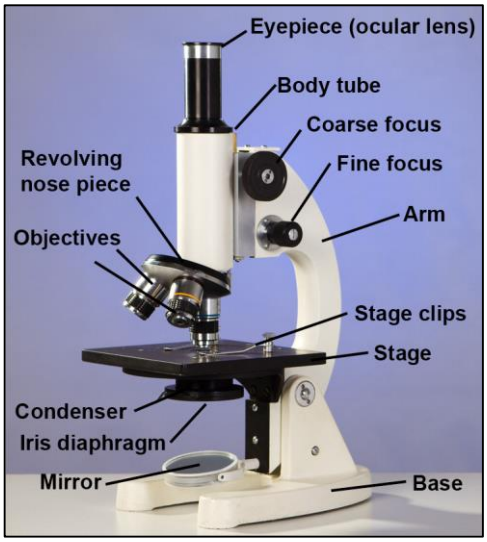
Objective	Magnification	Ocular lens	Total magnification
Scanning	4x	10x	40x
Low power	10x	10x	100x
High power	40x	10x	400x
Oil immersion	100x	10x	1000x

Below are images of typical light microscopes with parts labelled:

a) With built in light source



b) With mirror to direct an external light source



2. Context

- These instructions are for the use of science teachers, technicians and secondary school students who are under the direct supervision of a teacher.
- This SOP contains general guidelines only. Please consult the user manual for your particular microscope.

3. Safety notes

- Always carry the microscope with two hands. It is advisable to place one hand under the base of the box for extra support.
- If the microscope is stored in a box, always ensure the door is locked before picking up the box.
- If using sunlight as the external light source, never face the adjustable mirror directly into the sun.
- Never disassemble the microscope as doing so may damage it.
- Unplug from the power supply before replacing the bulb or moving the microscope.
- Never use coarse focus except with the scanning and low power objective lens. It is very easy to drive an objective through a slide.
- Make sure your workstation is set up ergonomically to use the microscope.
- Avoid prolonged use of the microscope. Take breaks to rest your eyes and make sure that the light intensity is not excessive.
- Make sure the microscope and any external light sources are regularly electrically tested and tagged.
- For all chemicals consult current SDS's.

4. Regulations, licences and permits

NA

5. Equipment

- Compound light microscope

- User manual for your microscope
- PPE: laboratory coat, enclosed shoes, hair tied back
- Microscope cover
- Prepared slides with coverslips
- Immersion oil (if using 100x objective)
- Light source (if required)
- Lens tissue and blower brush
- Lens cleaning fluid or Windex®. Do not use any other solvents on microscope lenses as they can loosen the glue used to hold the lens elements in place and ruin the lens.

6. Operating procedure

Set up and use of a compound light microscope

1. Read and be familiar with the user manual for your model of microscope.
2. Carry the microscope with two hands, one under the base and the other gripping the arm or frame.
3. Gently place the microscope on a flat, level surface and plug into a power source. Some microscopes have a built in light source but others have a mirror to focus natural light or an external light source.
4. With a built-in light source, turn on the light source and adjust the light setting so that it is not too bright by turning or sliding the brightness adjustment knob on the base.
5. 'If using an external light source direct the light via the mirror. Rotate the low power objective into position. Remove the eyepiece, look down the body tube and adjust the mirror and diaphragm setting so light is reflected up the tube and a circle of evenly illuminated light is visible in the field of view. Replace the eyepiece. Use the concave mirror side if the microscope has a fixed condenser lens or the flat mirror side if the microscope has an adjustable condenser'[1].
6. The iris diaphragm is located just above the light source on the bottom side of the stage. Using the lever attached, you can increase or decrease the amount of light reaching the specimen. Look through the eyepiece and adjust the sub-stage iris diaphragm to allow sufficient comfortable light through.
7. Between the stage and the iris diaphragm is the condenser. The condenser further aids in the focusing of the light onto the specimen. In some microscopes it can be moved up and down. To begin with, position it close to the stage. If you have a problem focusing your specimen then adjust the position of the condenser.
8. Adjust the stage down as low as possible with the coarse focus knob.
9. Begin by viewing the specimen with the lowest power objective lens in place and then increase to the higher power objective lenses.
10. Select the 4x scanning objective by rotating the nosepiece, ensuring it clicks into place.

(Operating procedure cont.…)

11. Place a prepared slide onto the stage and hold it in place with the metal clips. Centre it so that the specimen is under the objective lens. Move it with the stage control knobs either left to right or backwards and forwards.
12. After placing the slide on the stage look at the objective lens and the stage from the side and use the coarse focussing knob to bring the slide as close to the objective as possible without touching it.
13. Look in the eyepiece/s and slowly move the stage away from the objective lens with the coarse focusing knob. Stop when the image comes into view.
14. If using a binocular microscope adjust the distance between the eyepieces to suit your eyes by sliding the eyepieces in and out until you see one image. This is called the interocular distance.
15. Use the fine focus to sharpen the image. Scan the slide, select the part of the specimen you are interested in and center it in your field of view.
16. Adjust the sub-stage iris diaphragm to optimise the lighting.
17. Rotate in the low power 10x objective and refocus with the fine focus. You may need to open the iris diaphragm to let more light in. In general, the higher the power, the more light you require.
18. Repeat with the high power 40x objective, adjusting the iris diaphragm if required. Use only the fine adjustment knob to focus the microscope when using the higher power objective lenses.
19. If you have a 100x oil immersion objective, you will need to first focus on the specimen with the 40x objective. Next rotate the nosepiece so that a midway position is obtained between the 40x objective and the 100x objective. Place a small drop of immersion oil onto the slide coverslip then continue to rotate the nosepiece so that the 100x objective is rotated into the oil. The immersion oil should be used sparingly. **Never** use immersion oil with any of the other objectives. (N.B. It is possible to place the oil directly on a specimen that has been fixed or heat fixed and stained without a coverslip, e.g. bacterial slides. However, it is difficult to remove the oil from the slide without damaging the smear.) Any attempt to re-look at the slide with a low or high power objective may result in contamination of these objectives with the immersion oil. Do not use immersion oil on a wet mount unless you can secure the coverslip well.
20. Sharpen the image with the fine focus only and adjust the light with the iris diaphragm if required.
21. When finished, lower the stage, rotate the low power objective (4x) into position and remove the slide.
22. Clean the oil off the slide and the objective when finished with lens tissue and lens cleaning fluid. In order to return to work at the lower magnifications, the slide must be completely cleaned of any residual oil. Wipe the stage clean with a paper towel.
23. Turn off the light and at the main switch.
24. Report any problems to your teacher.
25. Cover the microscope with its dust cover.

(Operating procedure cont….)

Microscope handling and storage

1. When work is completed, lower the stage, remove the slide, rotate in the lowest power objective, wrap the cord loosely around the base and cover with a dust cover. Take care not to wrap the cord around a hot (built in) light source.
2. Always keep your microscope covered when not in use. Optics and mechanical parts must be protected from dust.
3. Always move the microscope with one hand under the base and the other hand gripping the arm or frame.
4. Keep microscopes away from vibration, moisture, high temperatures and direct sunlight.
5. Never store microscopes in chemical storage areas as corrosive fumes may damage metal and lenses.

Microscope maintenance

1. Treat lenses with great care as they can be easily scratched. Never use anything abrasive.
2. When cleaning lenses, first blow away any dust with a blower brush then use lens tissue and lens cleaning fluid such as Windex® to clean the objectives and eyepieces. Do not use paper towel or regular tissues, as they will scratch the lens. Do not use other solvents.
3. Do not remove eyepieces or objectives from their location but clean only their external surfaces.
4. Remove immersion oil from the 100x objective immediately after use with lens tissue and lens cleaning fluid.
5. Wipe dust off the body of the microscope with a damp cloth.
6. Never attempt to take a microscope apart. This could impair operation, efficiency and accuracy.
7. Have the microscope serviced regularly by a professional, as most microscopes require periodic lubricating and minor adjustment of their mechanical parts.
8. Follow your user's manual for instruction in replacing the bulb. Always allow a bulb to cool before replacing it. When replacing bulbs avoid touching the glass with your hands, use a tissue. Fingerprints can reduce bulb quality and reduce its life.

7. Trouble shooting/emergencies

- **First Aid:** If using immersion oil or lens cleaning fluid, please check the latest SDS for current first aid information prior to using.

COMMON FAULTS	POSSIBLE CAUSES
No light	• Power cord is not connected, power switch is off • Wrong bulb is installed • The bulb has burnt out • Light intensity control is turned down too low • Objective is not properly in position • If using the 100x objective immersion oil has not been applied
Image is too dark	• Increase light intensity • Sub-stage iris diaphragm is not open enough • Condenser is too low
Image is too light	• Decrease light intensity • Sub-stage iris diaphragm is open too much
Spot in the field of view that doesn't move when the slide is moved	Lens is dirty. Clean both the objective and eyepiece.
Poor image quality, poor resolution, image not sharp (100x oil objective)	• Clean objective, eyepiece and condenser • Check if immersion oil is contaminated or cloudy or air bubbles are present • Slide is wrong way up
Poor image quality, poor resolution, image not sharp (40x objective)	There is dirt or oil on the lens
Uneven illumination	• Adjust condenser • Make sure objective has clicked into place
Flickering light	• Bulb needs replacement • Loose connection at the outlet • Bulb not properly inserted • Check voltage supply
Half the viewing field is illuminated	Make sure the objective is clicked into place.
Unable to focus the slide	• Coverslip is too thick • Slide is the wrong way up • The stage is slowly dropping, adjust tension of course focus knob • Clean the slide, objective and eyepiece

8. Waste disposal

- Dispose of used lens cleaning tissue into the regular waste.
- Place used coverslips into a sharps container.
- Commercially prepared slides should be returned to the slide box.
- Used microscope slides from wet mounts can be washed and reused OR should be disposed of with broken glass. Slides with heat fixed smears are difficult to clean so are not reused and are disposed of with broken glass: refer to Science ASSIST AIS: Lab glass and porcelain disposal. (http://assist.asta.edu.au/resource/2395/ais-lab-glass-and-porcelain-disposal)

9. Related material

- Microscope User Manual specific to your microscope
- Risk assessment for use of the light microscope
- SDS for immersion oil
- SDS for lens cleaning fluid/Windex

Glossary:

Coarse focus – moves the mechanical stage to give approximate focus of the specimen.

Condenser – focuses light from the light source onto the specimen.

Condenser focus – adjusts the height of the condenser so that it focuses light from the light source onto the specimen.

Eyepieces or oculars – lenses that further magnify the image of the specimen produced by the objective lens (secondary magnification).

Field of view – the circular area of the specimen that you can see through the eyepiece.

Fine focus – moves the mechanical stage to give sharp focus of the specimen.

Immersion oil – an oil medium that has a high refractive index and is used with the 100x oil immersion objective to increase the resolution. Use immersion oil only for the purpose of microscopy.

Interocular adjustment – controls the distance between the two oculars to match the distance between the eyes of the user.

Magnification – is the degree of enlargement of the specimen.

Numerical aperture – a number written on the objectives that expresses the ability of a lens to resolve fine detail.

Objectives – lenses that produce primary magnification of the specimen.

Parfocal – allows the rotation from one objective to another with only fine focus adjustment required to focus the image.

Resolution – ability of a lens to distinguish and separate fine detail.

Stage – platform on which slides are supported for viewing.

Stage controls – allows movement left to right and backwards and forwards.

Sub-stage iris diaphragm – controls the amount of light entering the specimen.

Total magnification – magnification of the objective x magnification of the eyepiece.

Turret or nosepiece – holds the objectives.

Working distance – is the distance between the front lens of the objective and the specimen when it is focused. As magnification increases the working distance decreases.

References:

[1] WA Department of Education, 2010 *Science Laboratory Manual: Biology Techniques*, p 49. ©Department of Education (WA) (with permission)

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ATTACHMENT 2

ASSIST Information Sheets (AIS)

1. Decontaminate microbiological equipment
2. Microwave, pressure cooker or autoclave? Recommendations for best practice of sterilising agar
3. Preparing sterile equipment for microbiology

ASSIST INFORMATION SHEET:

Decontaminating microbiological equipment

Prompt and thorough decontamination of equipment used in microbiological activities is vital to protect staff, students and facilities from microbiological contamination. These procedures are suitable for the decontamination of Risk Group 1 microbes used in a Physical Containment level 1 laboratory.

Decontamination processes should commence during and immediately after a microbiology activity. Processes such as disposing of disposable items in bleach solution and wiping benches with 70% v/v ethanol after an activity is completed are implemented to contain any microbes and stop the transmission to students and staff in the following lab sessions.

Provide containers with relevant solutions and contaminated waste bags e.g. autoclave bags or oven bags for students to segregate and dispose of equipment and cultures. Each receptacle should be labelled with its function. Ensure these are removed from the laboratory at the end of the activity.

Procedure for using an autoclavable biohazard or oven bag for sterilising microbiological waste:

- **Loosely pack microbiological waste including agar plates into bags to no more than 2/3 full**. This will ensure that the steam during sterilisation will penetrate the entire load. Bags that are tightly filled to capacity will not allow effective steam penetration and the contents will not be sterilised even if all sterilisation parameters are met.
- **Make sure there are no sharp objects present** that may puncture the bag.
- **Loosely tape shut the bag leaving an opening of about 5–6cm** to allow good steam penetration. This can be done with autoclave tape or a rubber band. Never tightly close the bags as they are impervious to steam and therefore the temperature of the inside of the bag will not be sufficient for sterilisation.
- It is advisable to **place the bag into a secondary container** within the steriliser to prevent any leakage into the steriliser should the bag rupture. The container must be able to withstand the autoclaving conditions.
- **Do not overload the steriliser** with too many bags as this may block steam circulation.
- **Use a sterility compliance strip** to indicate if the correct time, temperature and pressure have been reached during the run time. These are available from scientific suppliers.
- **Sterilise at 15psi, 121^{o}C for 15–20 minutes.**
- After sterilisation has been verified, the autoclave or oven bag containing waste items should be **disposed of by placing it into a sturdy garbage bag which is sealed for immediate disposal in industrial bins.**
- **Wear heat protective gloves** when removing waste from the steriliser.

See table over the page for suggested decontamination techniques.

References

Society for General Microbiology. 2006. *Basic Practical Microbiology – A Manual.* Microbiology Online website, http://www.microbiologyonline.org.uk/file/ca2189fba3b39d24c5a44c1285d0082c.pdf

CONTAMINATED Item	Suggested decontamination technique
Inoculated agar plates – plastic	Pack unopened plates loosely in an autoclave bag, leaving an opening of about 5–6cm to allow good steam penetration. Autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Inoculated culture broth in McCartney or Bijou bottles.	Make sure that the lids are loose. Autoclave at 121°C, 15psi for 15–20mins. Empty contents in the sink with copious amounts of water. Wash in warm soapy water, rinse well and dry. *Resterilise:* Re-autoclave loosely lidded container. Store in a clean closed container.
Inoculated water in glass bottle	Make sure that the lids are loose. Autoclave at 121°C, 15psi for 15–20mins. Empty contents in sink. Wash in warm water and dry. *Resterilise:* Re-autoclave loosely lidded container. Store in a clean closed container.
Plastic dropping pipettes	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in the general waste.
Used swabs	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours or. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15-20 mins. When cool, place unopened autoclave bag in a garbage bag and dispose in the general waste.
Sterile 'L' spreader	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours, or 2) Place directly into an autoclave resistant container and cover with foil or place into an autoclave/oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15-20 mins. 3) After sterilisation, wash in warm soapy water, rinse and dry. *Resterilise:* Wrap in foil and sterilise in an autoclave or hot air oven. Store until required for re-use.
Sterile forceps	Carefully place into an autoclave resistant container such as a large test tube, cover with foil and autoclave. Wash in warm soapy water, rinse and dry. *Resterilise:* Wrap in foil or place inside a clean test tube, cover opening with foil and autoclave, Store until required for re-use.
Test tubes	Autoclave at 121°C, 15psi for 15–20mins. Empty contents in sink. Wash in warm soapy water, rinse and dry. *Resterilise:* Plug with non-absorbent cotton wool and autoclave or cover the opening of the test tubes with foil and sterilise in a hot air oven or autoclave. Store in a clean closed container.

CONTAMINATED Item	Suggested decontamination technique
Inoculating loop	Flame to red heat carefully in the blue flame of the Bunsen burner to prevent the transmission of aerosols. Cool and reuse immediately. Alternatively, if using disposable inoculating loops, 1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours.. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Susceptibility discs Mastrings (set of 6 or more antibiotic discs joined together)	Susceptibility discs and Mastrings should remain on the agar plate after examination. The agar plate remains closed. Pack unopened plates loosely in an autoclave bag and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Paper towel exposed to contaminated areas Used disposable aprons/lab coats Used gloves	If not soaked in bleach or alcohol, sterilise in an autoclave or pressure cooker. If soaked leave for the recommended time and then dispose of into the general waste. An autoclave or oven bag should be placed in the laboratory for students to place these waste items directly into the bag. Do not overfill the bag. Leave an opening of about 5–6cm to allow good steam penetration and sterilise for 15–20 min at 121°C and 15psi. Place the unopened autoclave bag into a sturdy garbage bag and seal for immediate disposal in an industrial bin.
Laboratory benches Plastic containers used for storage and distribution of equipment Any other hard surface	Dilute disinfectant in fresh water according to the manufacturer's instructions. Use in a spray bottle. Dilute ethanol to 70% in fresh water, use in a wash bottle. Ethanol is flammable. Apply liberally to laboratory bench or other hard surface to be decontaminated. Wipe lightly with paper towel. Allow the residual to air dry.

ASSIST AUSTRALIAN SCHOOL SCIENCE INFORMATION SUPPORT FOR TEACHERS AND TECHNICIANS

ASSIST INFORMATION SHEET:

Preparing sterile equipment for microbiology

Equipment used in microbiology should be sterile before using. This enables aseptic techniques to be used when transferring microorganisms for inoculation, sampling environmental areas, adding susceptibility discs to agar plates and Gram staining.

This equipment should be prepared before the class activity and stored in clean, lidded containers.

Equipment such as hockey stick spreaders, inoculating loops and sterile swab sticks can be purchased as single-use items from commercial scientific suppliers if the school budget allows or it is more time effective to do so.

In-house preparation of sterile items is cost effective to schools as some pieces of equipment can be repeatedly recycled. Care should be taken with ethanol as it is a flammable substance and should not be used near a naked flame.

Considerations:

- Sterilisation of equipment should be performed in a draught-free area.
- Items to be sterilised should be clean and dry, metal forceps should not be rusty, glass items should not have chips or cracks.
- Consult the planned activity or activities prior to sterilising items to ensure there is the required number of items available during the activity.
- Ensure the bench area for this purpose has been decontaminated with 70% ethanol prior to commencing.
- Soaking items in a container of 70% (v/v) ethanol for 10 minutes, disinfects/decontaminates, but does not sterilise items. Alcohols are not sporicidal.
- Aluminium foil or greaseproof paper may be used to wrap sterile items.
- Sterile items can be stored in a large lidded plastic container that has been decontaminated with ethanol and paper towel.
- **Glassware and metal instruments can be wrapped in aluminium foil and sterilised using dry heat in an oven at 160°C for 2–3 hours.**
- **All sterilising processes using an autoclave/steriliser or pressure cooker should be at 121°C for 15–20 minutes at 15psi (pounds per square inch of pressure).**
- Professional microbiologists and higher education providers promote the sterilisation technique of ‘flaming’ hockey stick spreaders and forceps prior to using by dipping in 70% ethanol and igniting it in the Bunsen flame. Incorrect techniques can encourage microbial aerosol transmission and risk the ethanol catching on fire. **Science ASSIST does not recommend this practice in the school setting, but instead recommends sterilising these items in an autoclave or an oven.**

Item	Suggested sterilising technique	Alternative technique
Sterile plastic Petri dishes	Purchase sterile, leave wrapped in original packaging until required. (Do not autoclave prior to use. Plates do not retain shape when autoclaved.)	
Sterile glass Petri dishes	Wrap glass Petri dishes in greaseproof paper or aluminium foil and sterilise in an autoclave	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2–3 hours
Nutrient agar plates	Prepare agar solution according to the manufacturer's instructions, autoclave in a heat-safe bottle with lids loose and pour plates when temperature of sterile agar is ~50^0C using aseptic technique. When set, wrap in plastic wrap. Store at 4°C until required. See ASSIST SOP: Preparing agar plates	Purchase prepared and sterile from a biological supplier
Nutrient broth	Prepare broth solution according to the manufacturer's instructions. Aliquot ~15mL into McCartney bottles (28mL capacity) keep lids loose. Autoclave. When cool tighten lids and store at 4°C until required.	Purchase prepared and sterile from a biological supplier
Sterile water	Aliquot 2mL into Bijou bottles (7mL capacity) keep lids loose. Autoclave. When cool tighten lids and store at 4°C until required.	
Sterile plastic dropping pipettes	Purchase single-use pipettes from commercial scientific, biological or medical suppliers.	
Sterile swab stick	Purchase sterile, leave wrapped in original packaging until required.	Autoclave cotton buds in foil covered beaker.
Sterile 'L' spreader	Wrap in aluminium foil and autoclave. Store until required.	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2-3 hours.
Sterile forceps	Wrap in aluminium foil or place inside a clean test tube, cover opening with aluminium foil and autoclave. Store until required.	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2-3 hours.
Sterile test tubes/ conical flasks	Cover opening with foil or plug with non-absorbent cotton wool. Autoclave.	Cover opening with aluminium foil. Sterilise using dry heat in an oven at 160°C for 2–3 hours
Inoculating loop	Flame to red heat in the blue flame of the Bunsen burner.	Purchase sterile disposable inoculating loops, leave wrapped in original packaging until required.

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ASSIST AUSTRALIAN SCHOOL SCIENCE INFORMATION SUPPORT FOR TEACHERS AND TECHNICIANS

ASSIST INFORMATION SHEET:

Microwave, pressure cooker or Autoclave? Recommendations for best practice of sterilising agar.

Preparation of agar plates is central to effectively and economically expose our students to the wonderful world of micro-organisms. School science labs have long questioned the best physical method of control to sterilise agar prior to pouring into Petri dishes. Methods used for sterilizing include pressure cookers, sterilisers or autoclaves. Most schools have limited financial resources available, and sourcing processes and equipment that are cost effective is a priority. So which method should you choose and why?

Sterility of processes and equipment forms the very essence of good microbiological laboratory practice and the teaching of these methods to students. This includes the preparation and presentation of agar plates and other equipment used either before, during or after the practical has been run. Teaching aseptic technique to students can be part of the unit of learning; however can be a difficult process to master for first timers. Outcomes of student practicals may be misleading or incorrect if external contaminates are introduced at any stage prior to or during inoculation. Unfortunately, this is often not evident until much time and effort by the student and lab technician has passed and the results are interpreted. Ascertaining the source of any contaminant can be difficult and inconclusive.

While many types of agar plates are available commercially, many laboratory technicians prepare plates in-house thus reducing the cost to schools. Although it can be time consuming, many schools find the cost benefit far outweighs time spent. Many school science preparation areas are used for many disciplines of science and are not usually specific to microbiology or necessarily a 'clean' area. Schools may be permitted by their state jurisdictions to use micro-organisms from Risk Group 1* and/or culture environmental samples. It is impossible to predict what, if any, further contaminants may be introduced to an agar plate during a practical session. Hence the need to 'get it right' by initial effective sterilisation of agar to ensure plates distributed to students do not contain any contaminants.

Universally, micro-organisms are ubiquitous. Effective sterilisation of a liquid such as agar is achieved when all viable organisms are eliminated[1]. The most effective and suitable method of sterilising agar is by using moist heat in the form of steam under pressure i.e. 121^{o}C for 15 minutes at 15 pounds per square inch (psi). This method will denature & coagulate enzymes and other cell constituents in the bacterial cell. Sterilization can be guaranteed only when these parameters are reached.

Sterilisation of agar and plates is usually done in an autoclave or a commercially available pressure cooker with a gauge and the capacity to reach 15 psi, which provides these conditions. Microwave ovens will not sterilise as they do not provide these conditions and therefore are not a suitable alternative to a pressure cooker or autoclave. Water boils at 100^{o}C at atmospheric pressure, but if pressure is raised, the temperature at which the water boils also increases. In an autoclave or pressure cooker the water is boiled in a closed chamber. As the pressure rises, the

boiling point of water also raises. At a pressure of 15 psi inside the autoclave, the temperature is said to be 121°C. Exposure of articles to this temperature for 15 minutes sterilises them.[2]

Due to the action of a microwave oven, micro-organisms will not be killed. Microwaves penetrate unevenly and there are also 'hot spots' caused by wave interference. The whole heating process is different because you are 'exciting atoms' rather than 'conducting heat'.[3] The heat and pressure required to effectively sterilise agar will be insufficient and cannot be maintained for the required period of time. The agar will boil over before any of the required parameters are reached.

As laboratory technicians, we are constantly on the lookout for more efficient ways of finding good quality relevant resources for our students and teachers within budgetary constraints. Sourcing equipment such as a pressure cooker or autoclave is important to ensure the validity of student results and is imperative for microbiological safety.

***WHO Risk Group 1** (no or low individual and community risk). A micro-organism that is unlikely to cause human disease or animal disease (AS 2243.3)

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[1] Todar, Kenneth 2008, 'Control of Microbial Growth', Todar's Online Textbook of Bacteriology http://textbookofbacteriology.net/control_1.html (Accessed 01/04/2014)

[2] Rao, Sridhar 2008 'Sterilization and Disinfection', Department of Microbiology, JJMMC, Davangere www.microrao.com/micronotes/sterilization.pdf (Accessed 01/04/2014)

[3] Brain, Marshall 'How microwave cooking works', howstuffworks.com, http://home.howstuffworks.com/microwave2.htm (Accessed 01/04/2014)

ATTACHMENT 3

Laboratory rules – Microbiology

Laboratory rules – Microbiology

- Science ASSIST strongly recommends that all science rooms be locked unless a teacher or other authorised person is present.
- Students must be supervised at all times in the laboratory. Reckless or irresponsible behaviour will not be tolerated
- Eating, drinking, smoking, and shaving and the application of cosmetics are prohibited.
- Storage of food and drink in the laboratory refrigerators is prohibited.
- Work benches must be decontaminated prior to starting work, following spills and when work is completed.
- Precautions must be taken to ensure reading and writing materials do not become contaminated.
- No equipment or materials are to be removed from the laboratory.
- Appropriate Personal Protective Equipment (PPE) must be worn at all times in the laboratory. This includes:
    - Properly fastened laboratory coat or disposal apron
    - Non-slip shoes that cover the toes, upper surface of the foot and the heel.
    - Approved protective eyewear must be worn
    - Appropriate gloves should be worn to suit the particular application
    - Long hair must be tied back
- Laboratory coats must be removed before leaving the laboratory
- Hands must be thoroughly washed at the completion of a task or before leaving the laboratory.
- Inform the teacher or laboratory technician immediately in the event of a spill or breakage.
- Report all accidents, hazards, incidents or injuries to your teacher.
- Do not commence work unless a site-specific risk assessment is completed, and control measures are implemented.
- All microbiological samples should be regarded as potentially hazardous or infectious. Safe handling procedures must be implemented for all hazardous substances or procedures
- A laboratory safety induction must be completed before undertaking any work in a laboratory.
- Cultures must be clearly identified, dated and appropriately stored.
- Care must be taken to minimise the production of aerosols.
- Care must be taken to prevent the dissemination of material while flaming a wire loop.
- Mouth pipetting is prohibited.
- Laboratory waste must be decontaminated prior to disposal. Wastes must not be poured down sinks or drains.
- Your work space should be left clean and tidy at the end of the practical session. Bench surface should be decontaminated before leaving the lab.

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